Blood morphology matters: Common clinically significant findings haematology numbers (and dot plots) might miss.

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November 2024

Disclosure:

I am an employee of IDEXX Laboratories Ltd.

Disclaimer:

The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions. Diagnosis and treatment decisions are the ultimate responsibility of the primary care veterinarian.

Presentation overview:

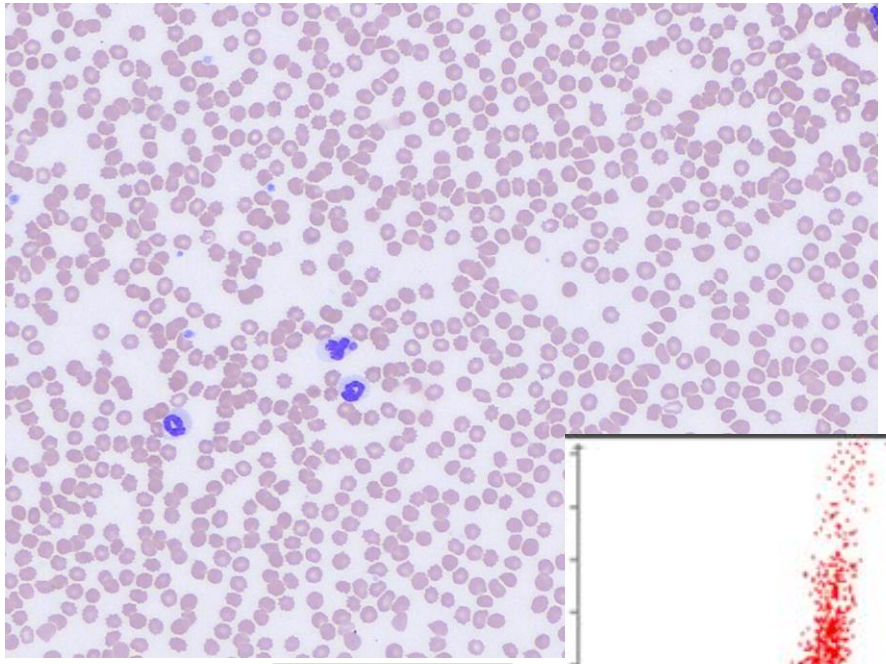
- Understand common limitations of the automated haematology counts to critically assess results
- Recognize common and significant changes in the blood smear morphology that are not identified by the haematology analysers
- Integrate information from the blood smear exam with the information from the haematology analyser in the clinical case context

Haematology is an important diagnostic tool

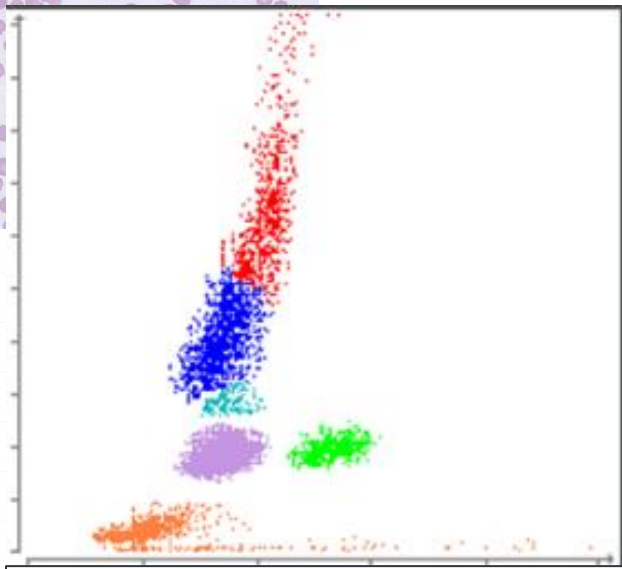
- Part of minimal data base
- Investigation of every sick patient

Components of haematology results

- Automated analysis
  - Numerical values
  - Dot plot analysis
- Blood film evaluation



Neutrophils	*19.68	2.95 - 11.64 x10^9/L
Lymphocytes	*1.29	1.05 - 5.10 x10^9/L
Monocytes	*0.70	0.16 - 1.12 x10^9/L
Eosinophils	*0.78	0.06 - 1.23 x10^9/L
Basophils	*0.08	0.00 - 0.10 x10^9/L
Nucleated RBC	*Suspected	
Platelets	*533	148 - 484 x10^9/L
PDW	--,--	9.1 - 19.4 fL
MPV	18.7	8.7 - 13.2 fL
Plateletcrit	1.00	0.14 - 0.46 %



ProCyte Dx - Canine
Normal WBC Dot Plot

…so much data!

RBC	3.75	5.65 - 8.87 x10^12/L	
Haematocrit	0.265	0.373 - 0.617 L/L	
Haemoglobin	94	131 - 205 g/L	
MCV	70.7	61.6 - 73.5 fL	
MCH	25.1	21.2 - 25.9 pg	
MCHC	355	320 - 379 g/L	
RDW	18.0	13.6 - 21.7 %	
% Reticulocyte	3.1	%	
Reticulocytes	114.8	10.0 - 110.0 K/µL	
Reticulocyte Haemoglobin	26.7	22.3 - 29.6 pg	
WBC	54.21	5.05 - 16.76 x10^9/L	
Neutrophils	*0.82	2.95 - 11.64 x10^9/L	
Bands	*Suspected		
Lymphocytes	*10.93	1.05 - 5.10 x10^9/L	
Monocytes	*42.44	0.16 - 1.12 x10^9/L	
Eosinophils	0.01	0.06 - 1.23 x10^9/L	
Basophils	0.01	0.00 - 0.10 x10^9/L	
Platelets	*32	148 - 484 x10^9/L	
PDW	- --.--	9.1 - 19.4 fL	
MPV	17.2	8.7 - 13.2 fL	
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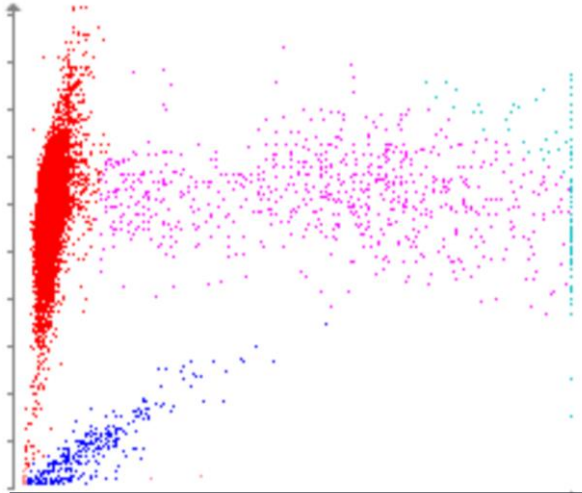
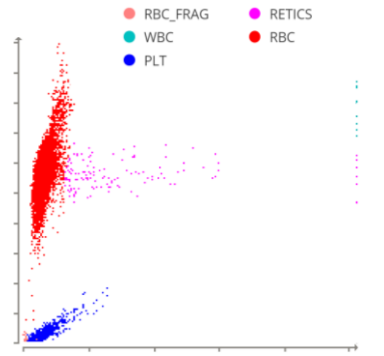
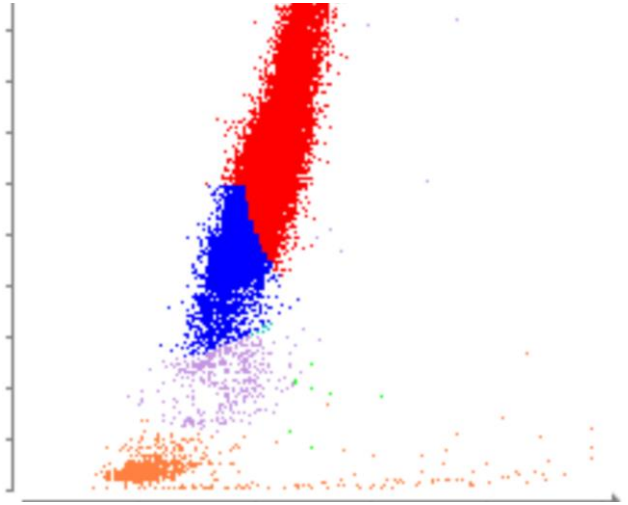


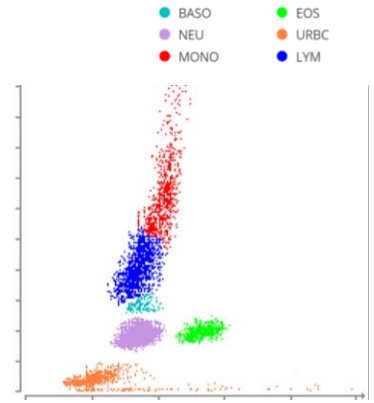
ProCyte Dx – Canine Patient RBC Dot Plot



ProCyte Dx - Canine Normal RBC Dot Plot



ProCyte Dx – Canine Patient WBC Dot Plot

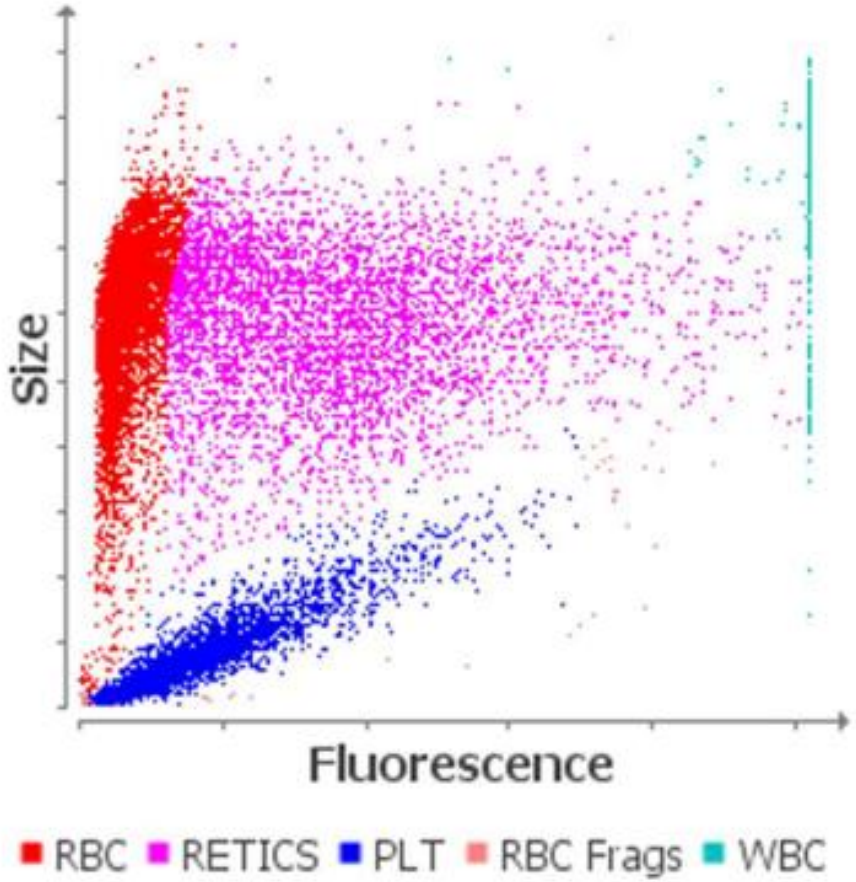


ProCyte Dx - Canine Normal WBC Dot Plot

… and many sample features that interfere with automated CBCs

- Clots
- Platelet clumps
- Macroplatelets
- RBC agglutination
- nRBC
- Heinz bodies
- Lipemia
- Leukocyte agglutination
- Delay in sample handling (increase in MCV, haemolysis, changes in leucocytes, etc)

Why is morphological evaluation of cells important?

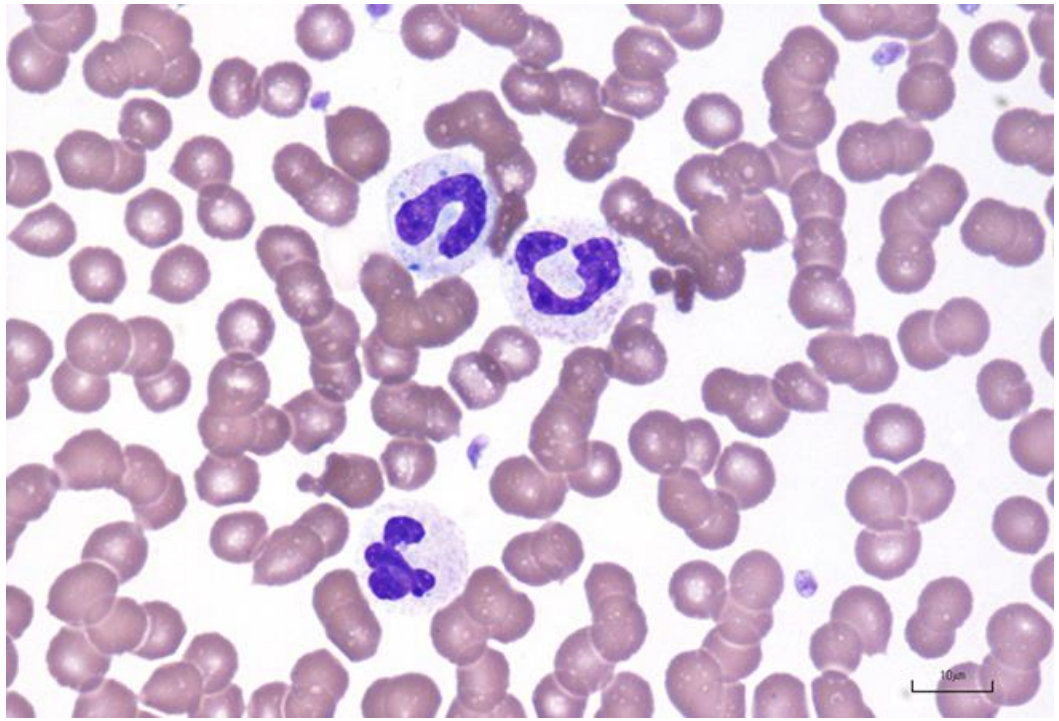


ProCyte Dx – Canine patient RBC Dot Plots

Bands	*Suspected

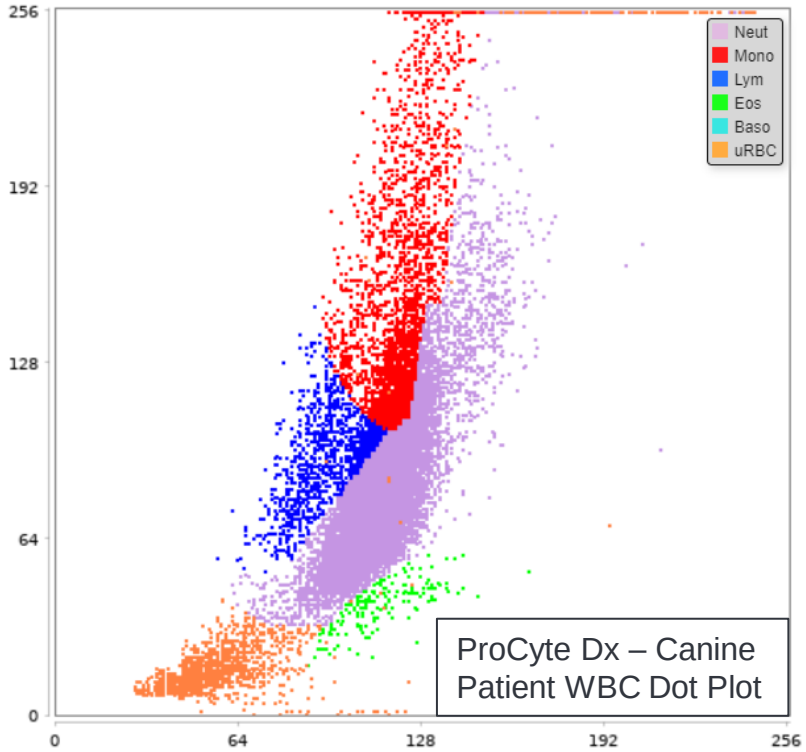
* Confirm with dot plot and/or blood film review.

Immature and/or toxic neutrophils likely present - consider inflammation



When should blood cell morphology be reviewed ?

- Ideally all smears should be evaluated
- Investigation of critically ill patient
- Moderately to severely anaemic patients
- Markedly high WBC counts
- Markedly low WBC/Plt counts
- Flags in dot plots/indistinct separation of clusters
- Unexpected or suspicious instrument results



Bands	* Suspected

* Confirm with dot plot and/or blood film review.

Immature and/or toxic neutrophils likely present - consider inflammation

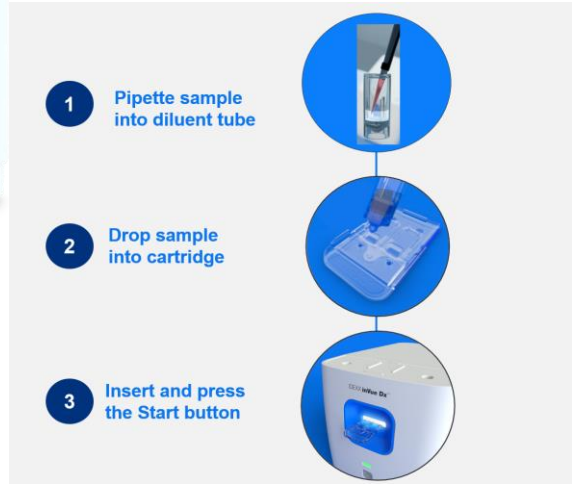
ASVCP guidelines

Some suggested numerical guidelines for medical review of blood smear and CBC data

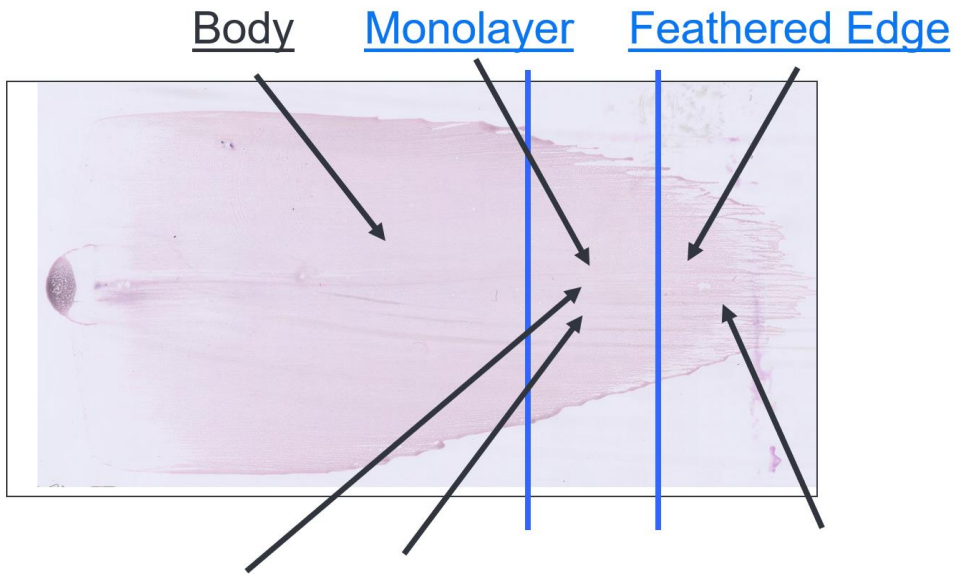
	CRITERIA
WBC	Leukopenia < 3 x10e9/L
	Leukocytosis > 30 x10e9/L
	Lymphocytosis > 10 x10e9/L
	Any unclassified cells
Platelets	Thrombocytopenia < 100 x10e9/L
	Thrombocytosis > 900 x10e9/L
	Abnormal MPV (if reported by instrument)
RBC	Moderate to severe anaemia
	>5 nRBC/100WBC
	Abnormal MCV

Modified from ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. ASVCP, 2013.

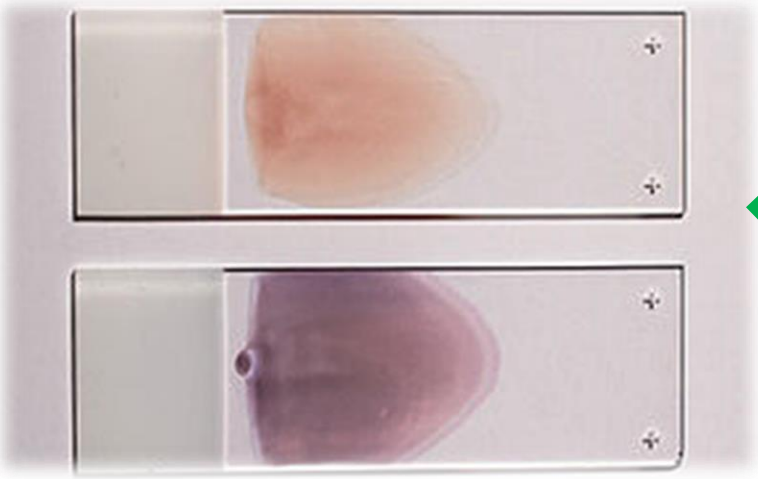
Morphological evaluation of blood cells

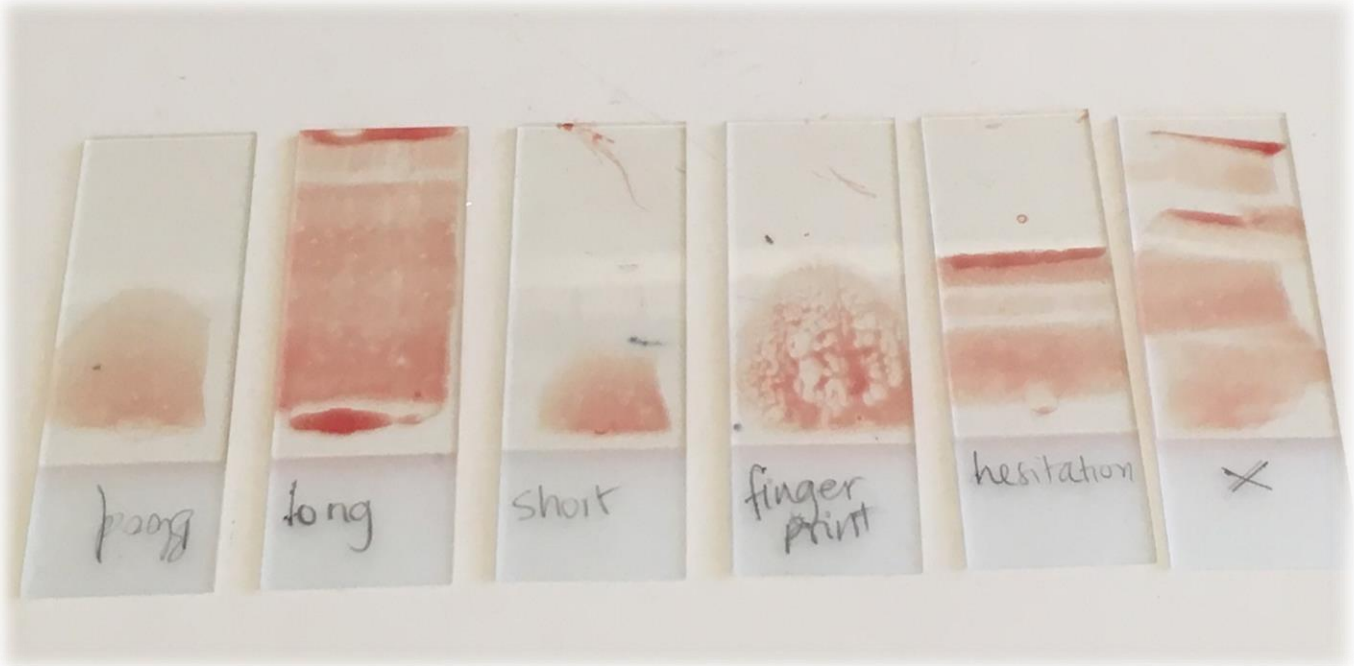


Blood smear



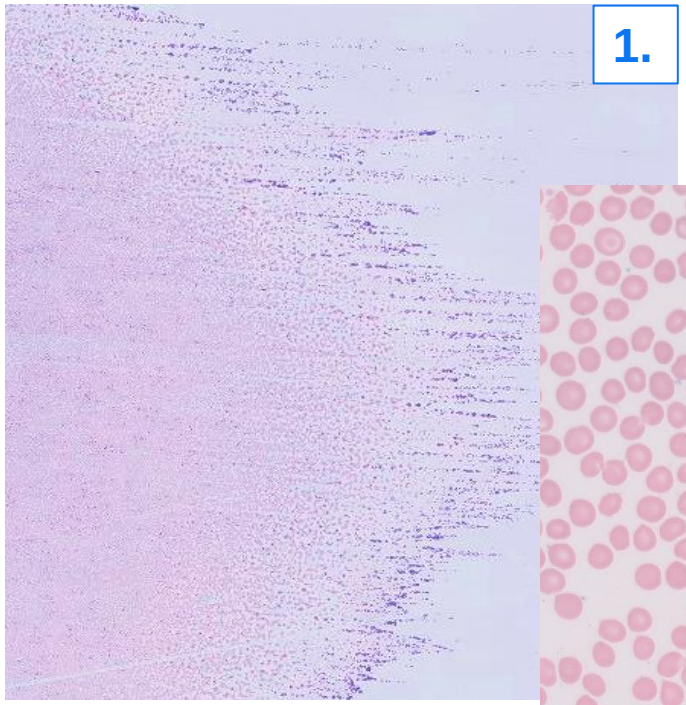
- Rouleaux
- Agglutination
- Estimate numbers
- Morphology
- Platelet clumps
- Large cells
- Microfilaria

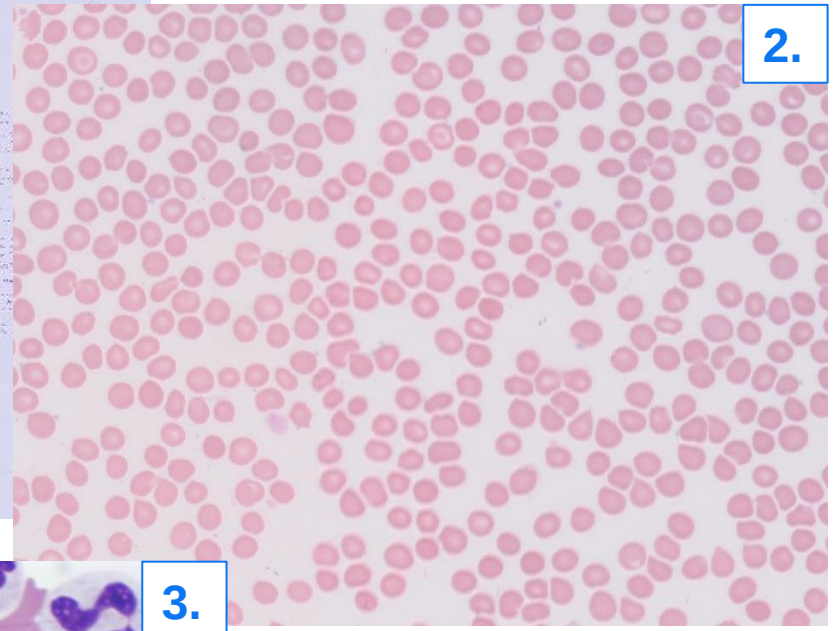


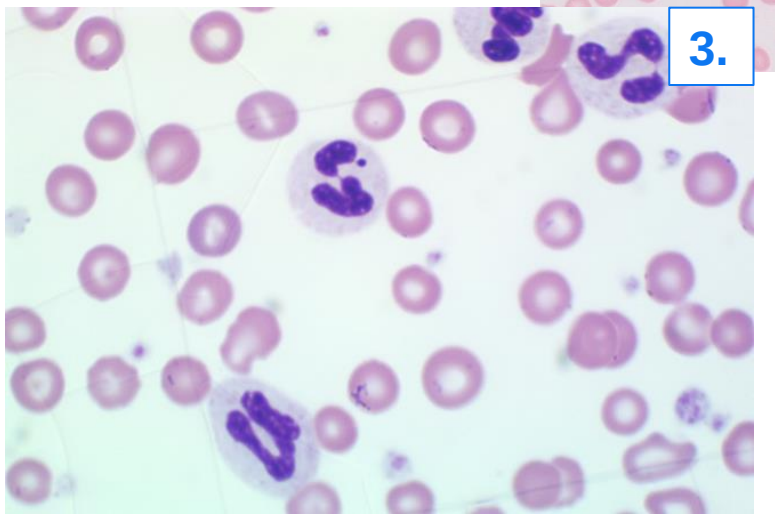


Systematic approach to blood smear review

1. Start small – with low magnification (10x) and then feathered edge
2. Go deeper - Go two to three fields back to the body of the smear into the monolayer
3. Go bigger – Increase to oil and evaluate morphology
4. Have a consistent approach:
   + Platelets
   + Erythrocytes
   + Leukocytes







Blood smear evaluation steps - Systematic stepwise approach

Cell distribution

For all cell types:

Agglutination/ aggregation

Platelets

Estimate plt numbers

Examine morphology

Red Blood cells

Evaluate:

- size,
- shape,
- color,
- inclusions.

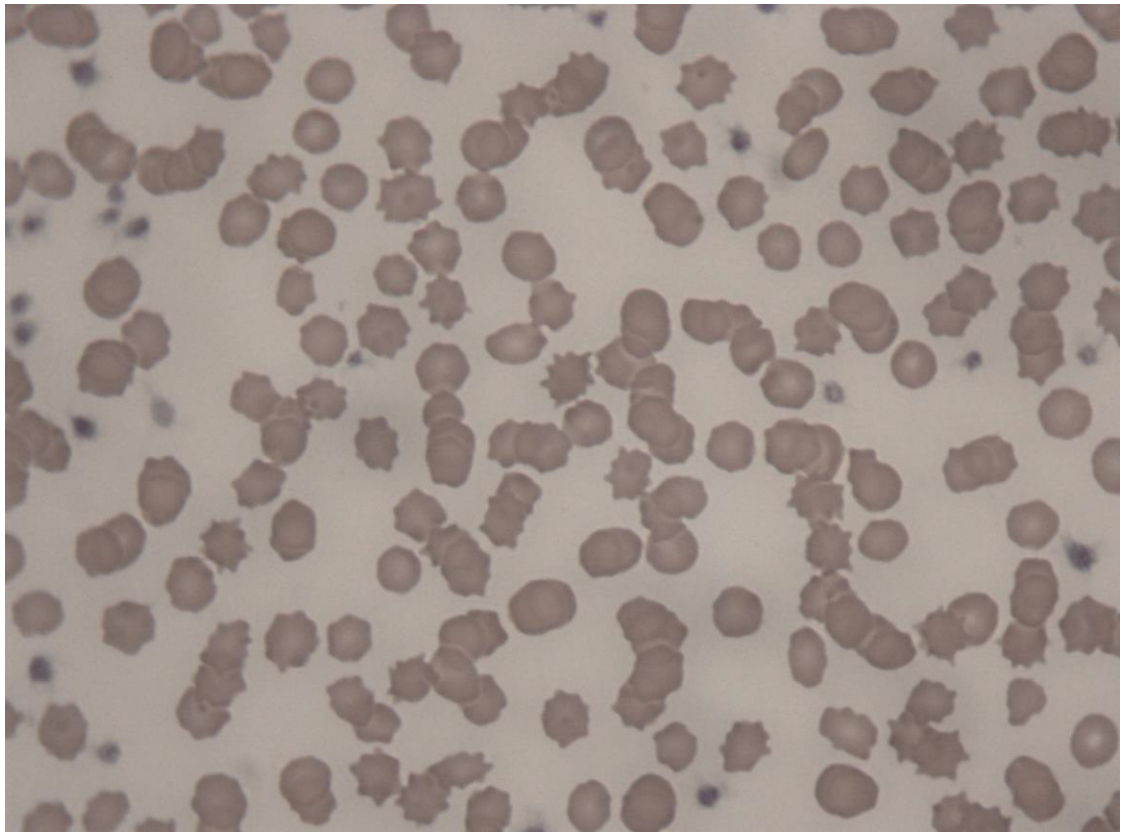
White blood cells

Do or verify differential cell count

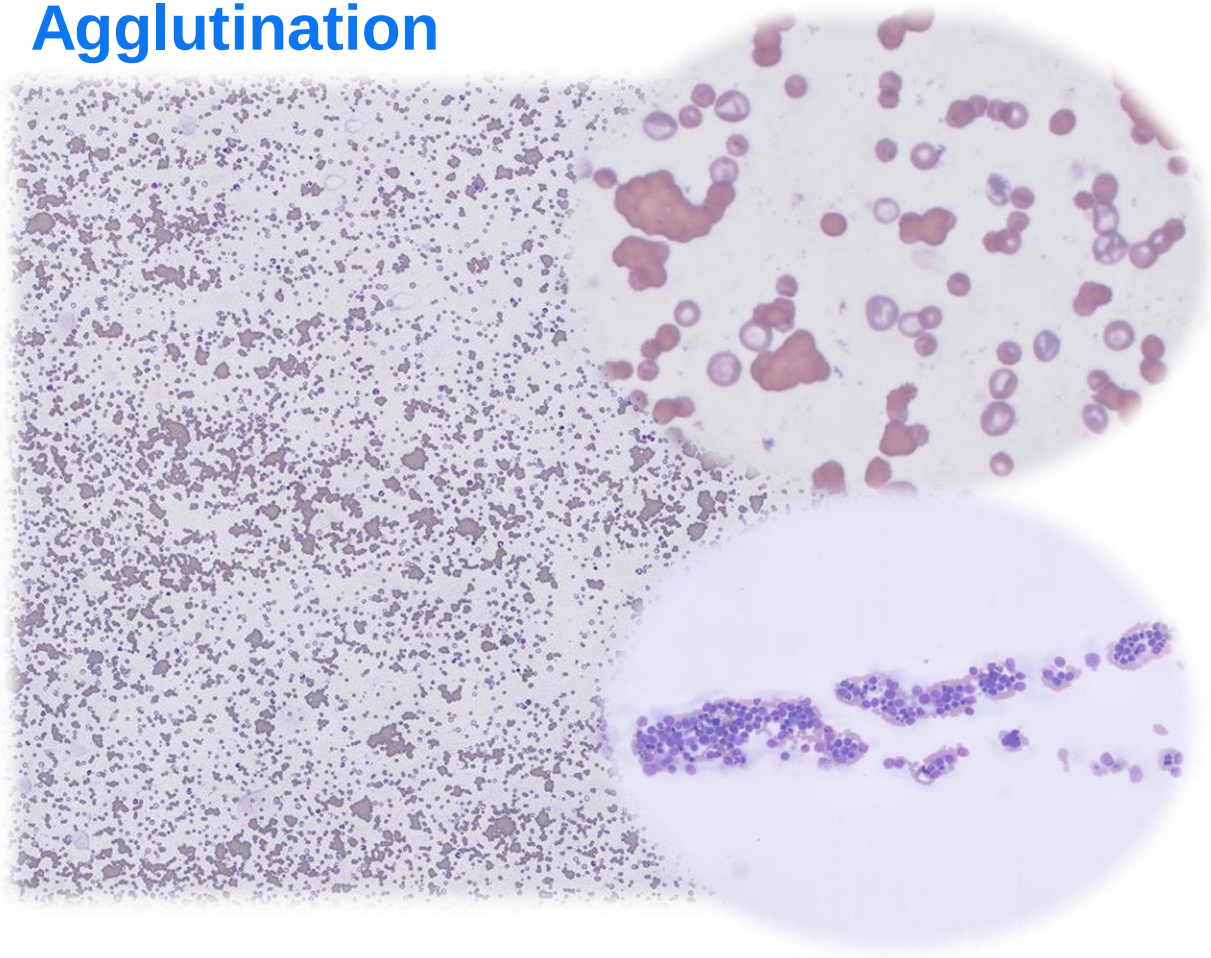
Evaluate morphology changes and inclusions

Cell distribution

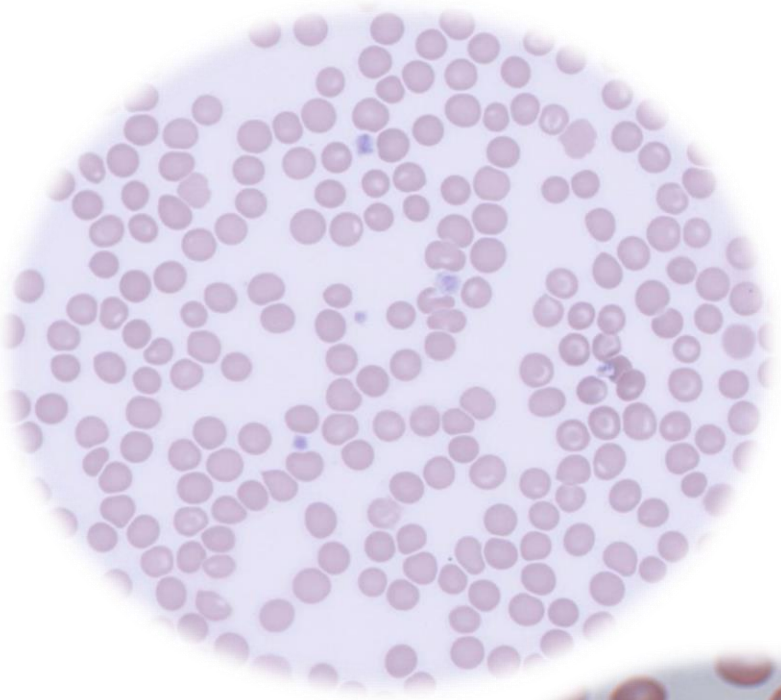
Rouleaux

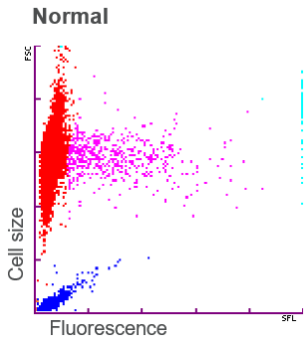


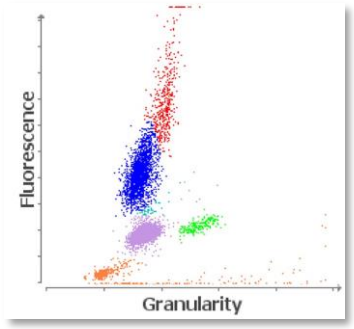
Agglutination

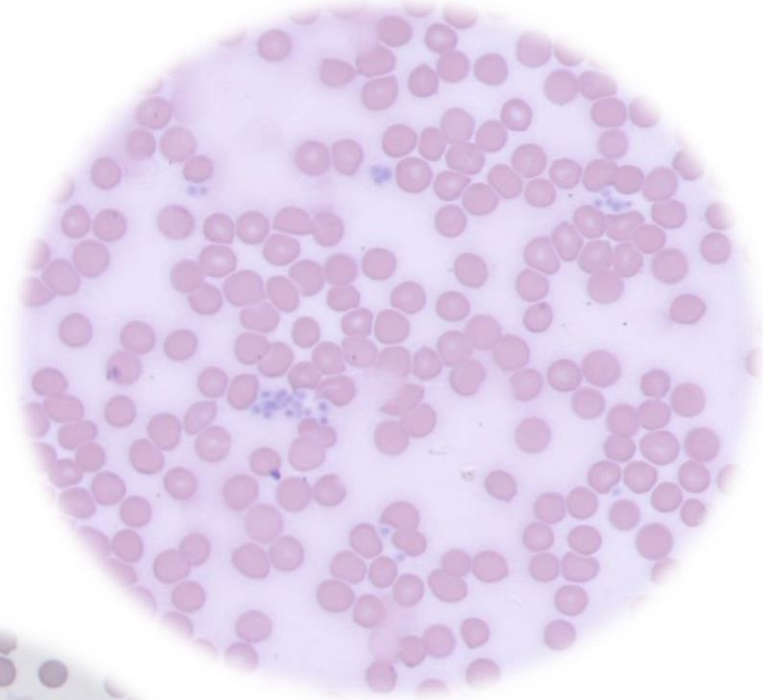


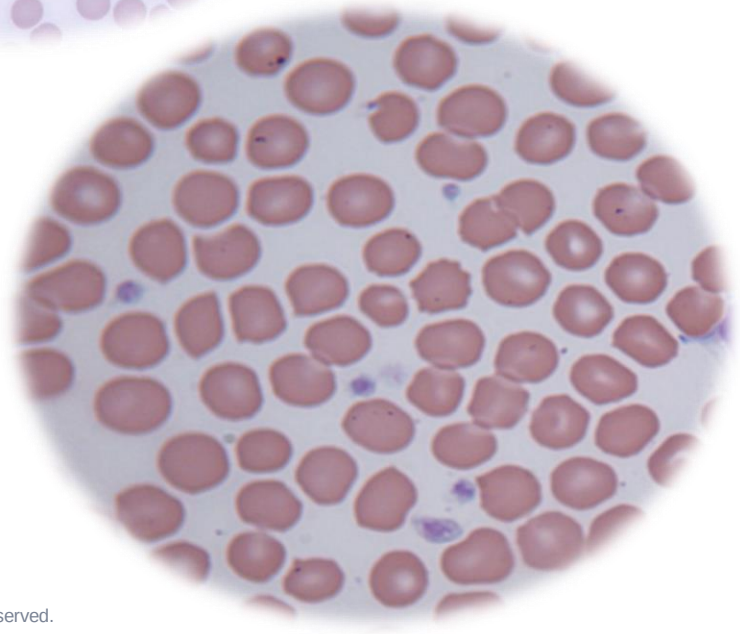
What is normal?
PLT

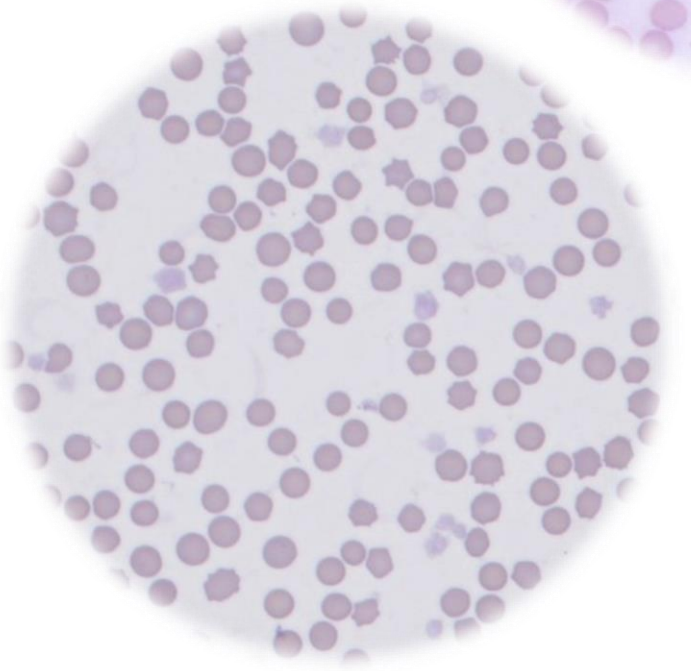




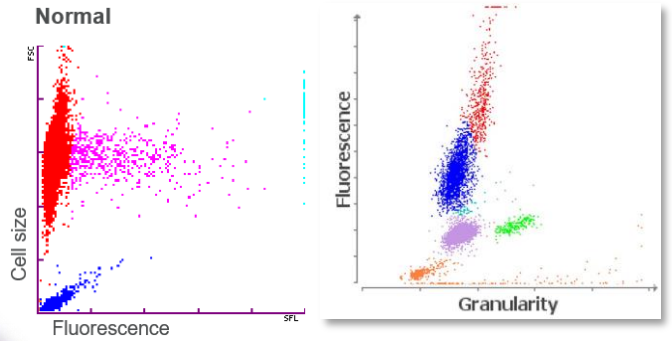


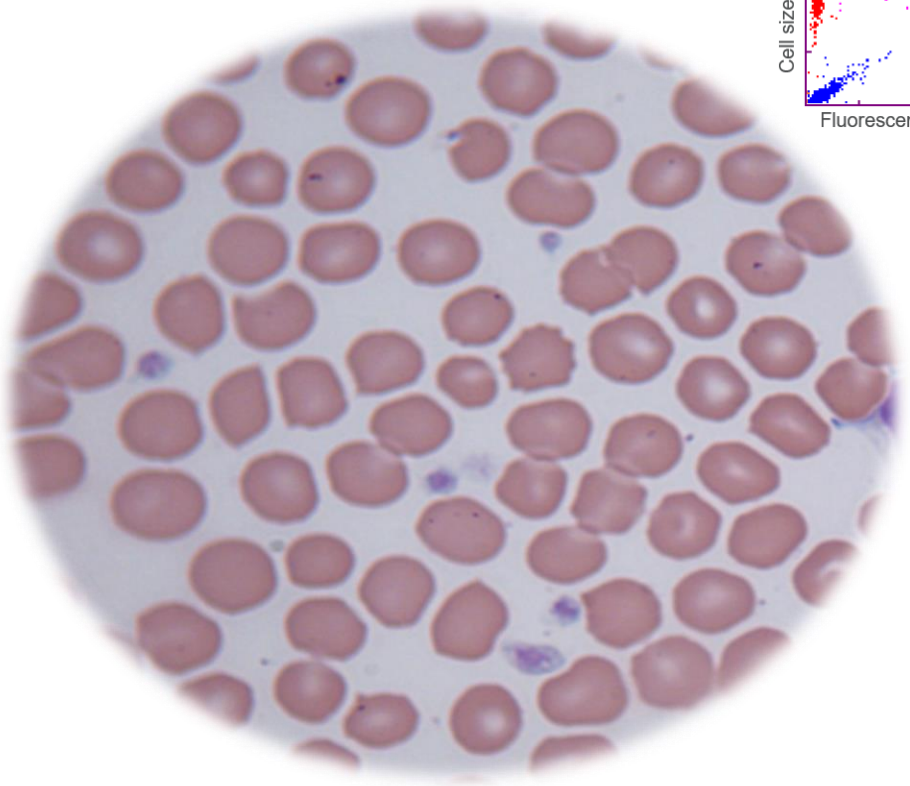


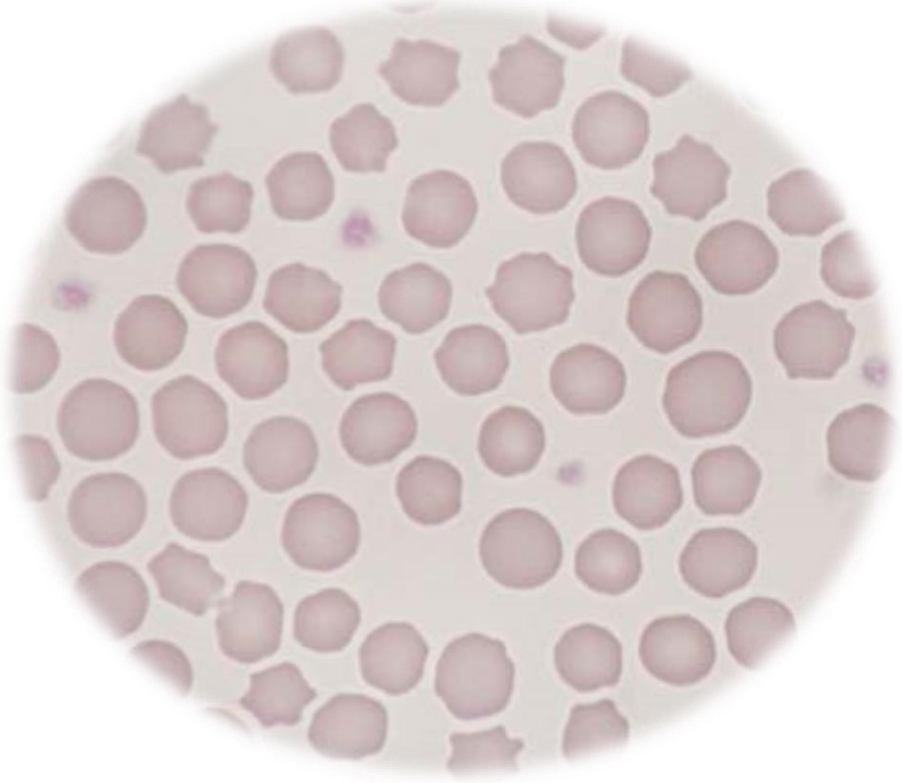




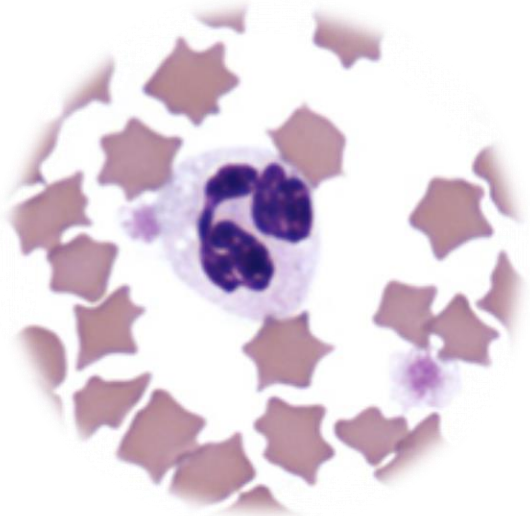
What is normal?
RBC

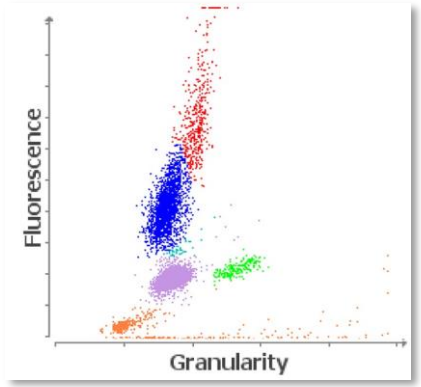


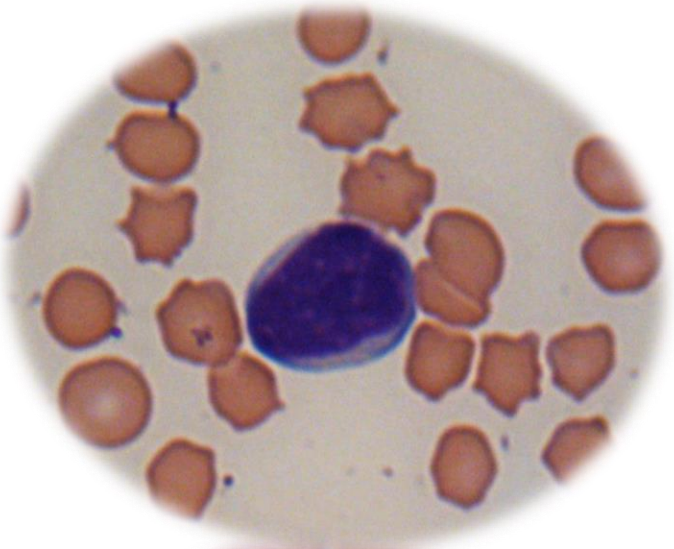


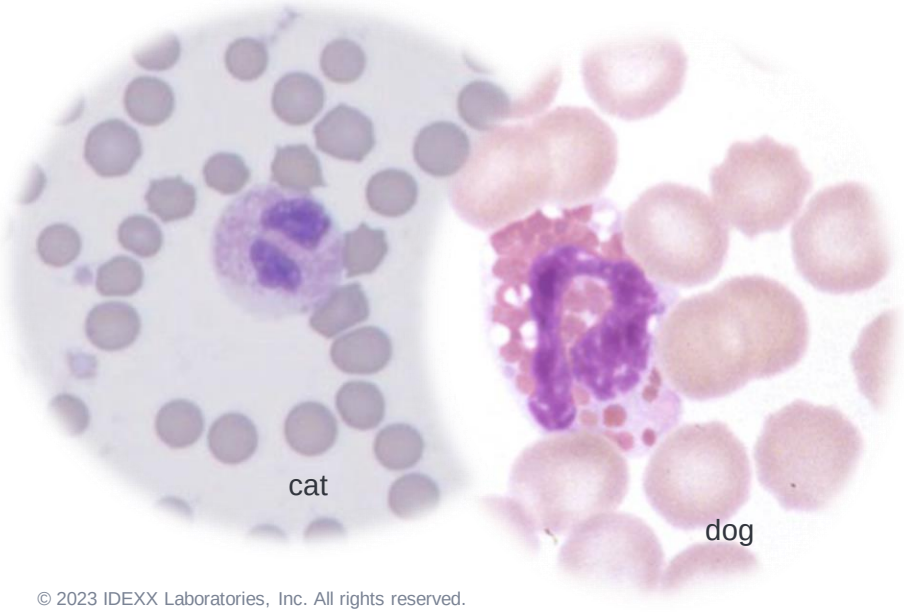


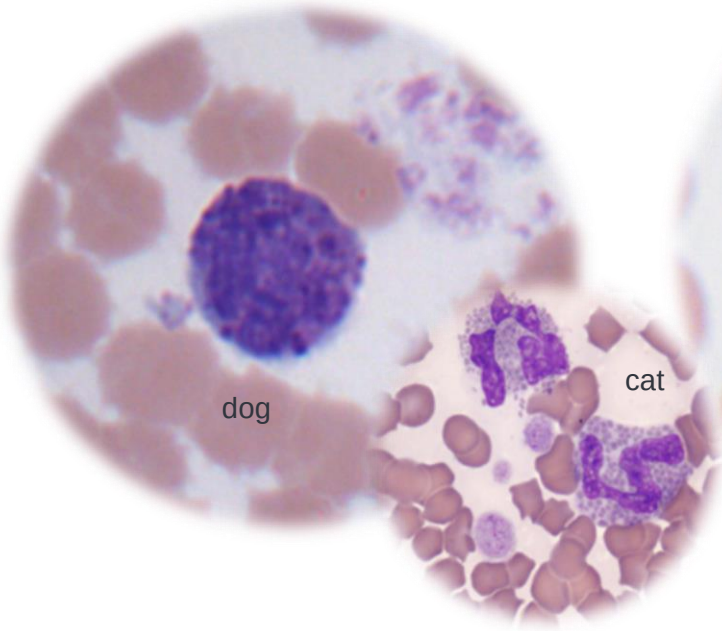
What is normal?
WBC

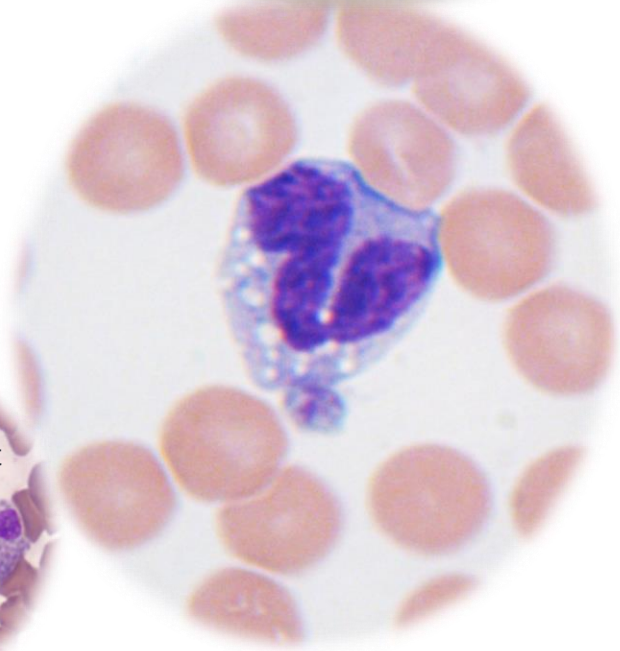






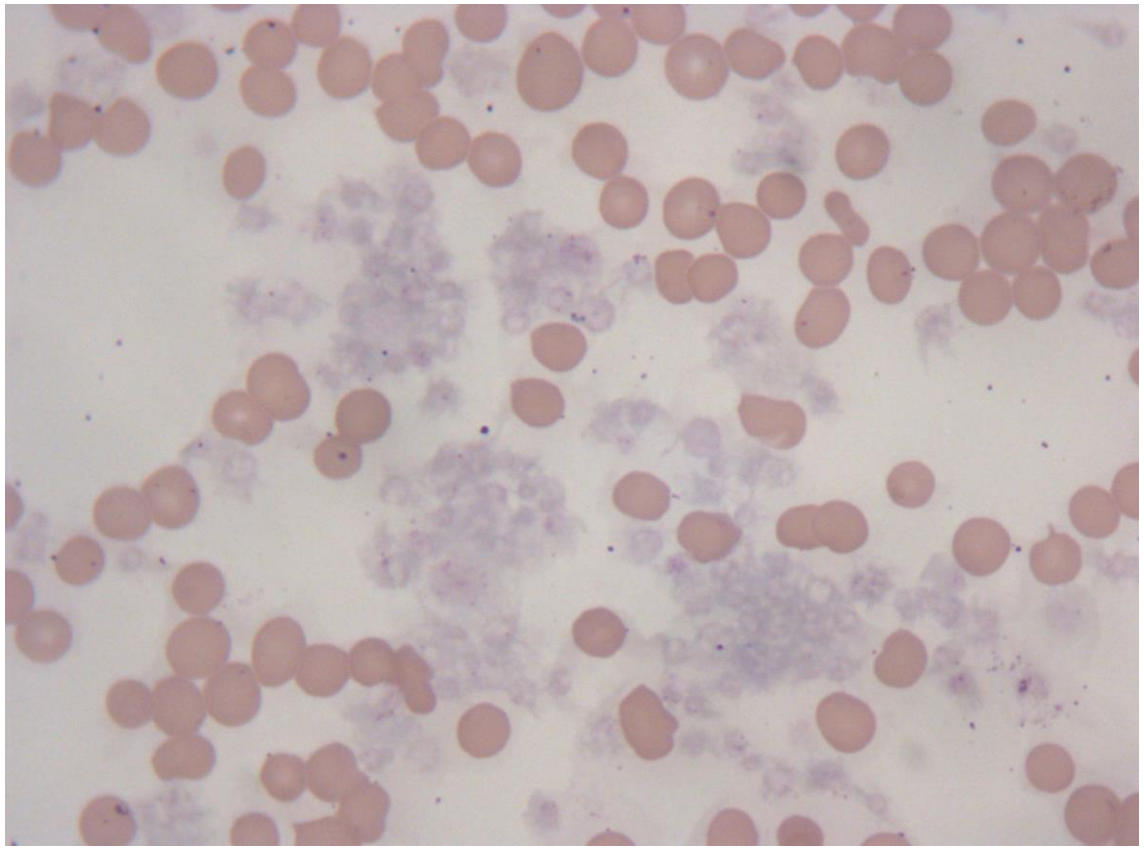




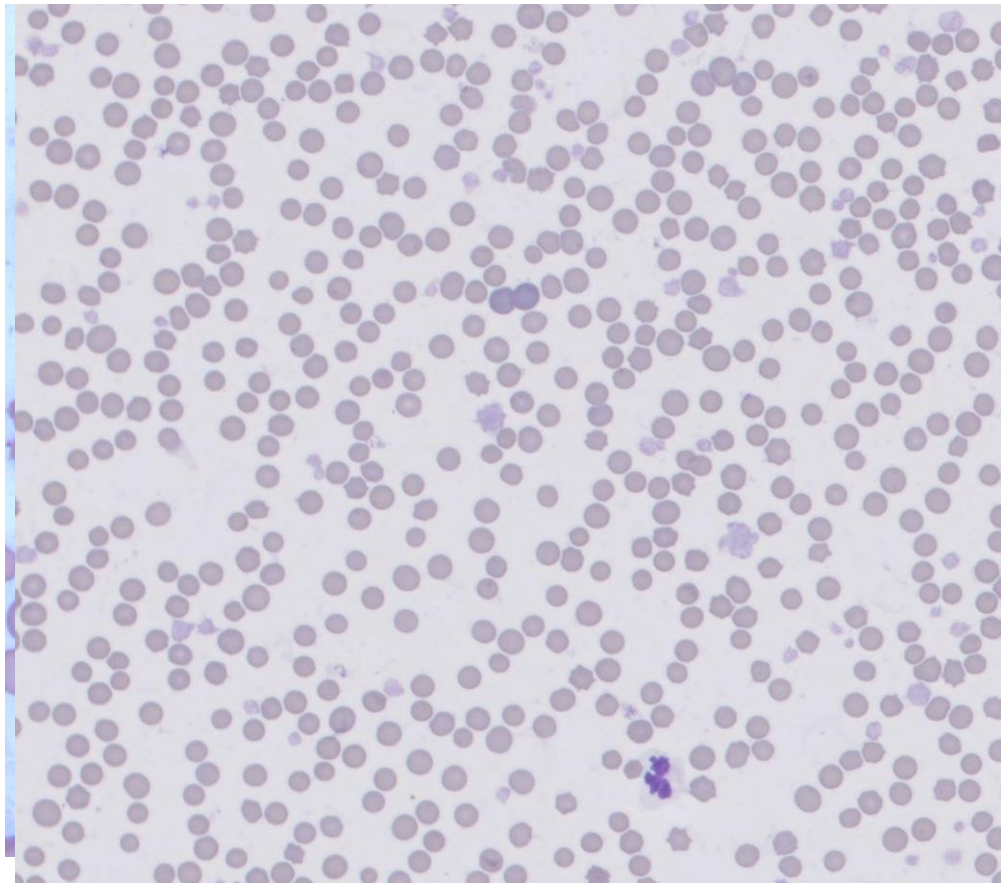


Platelets

Platelet clumps



Macroplatelets



Platelet estimates

- ✓ Assuming NO PLT CLUMPS in feathered edge and NO CLOTS!

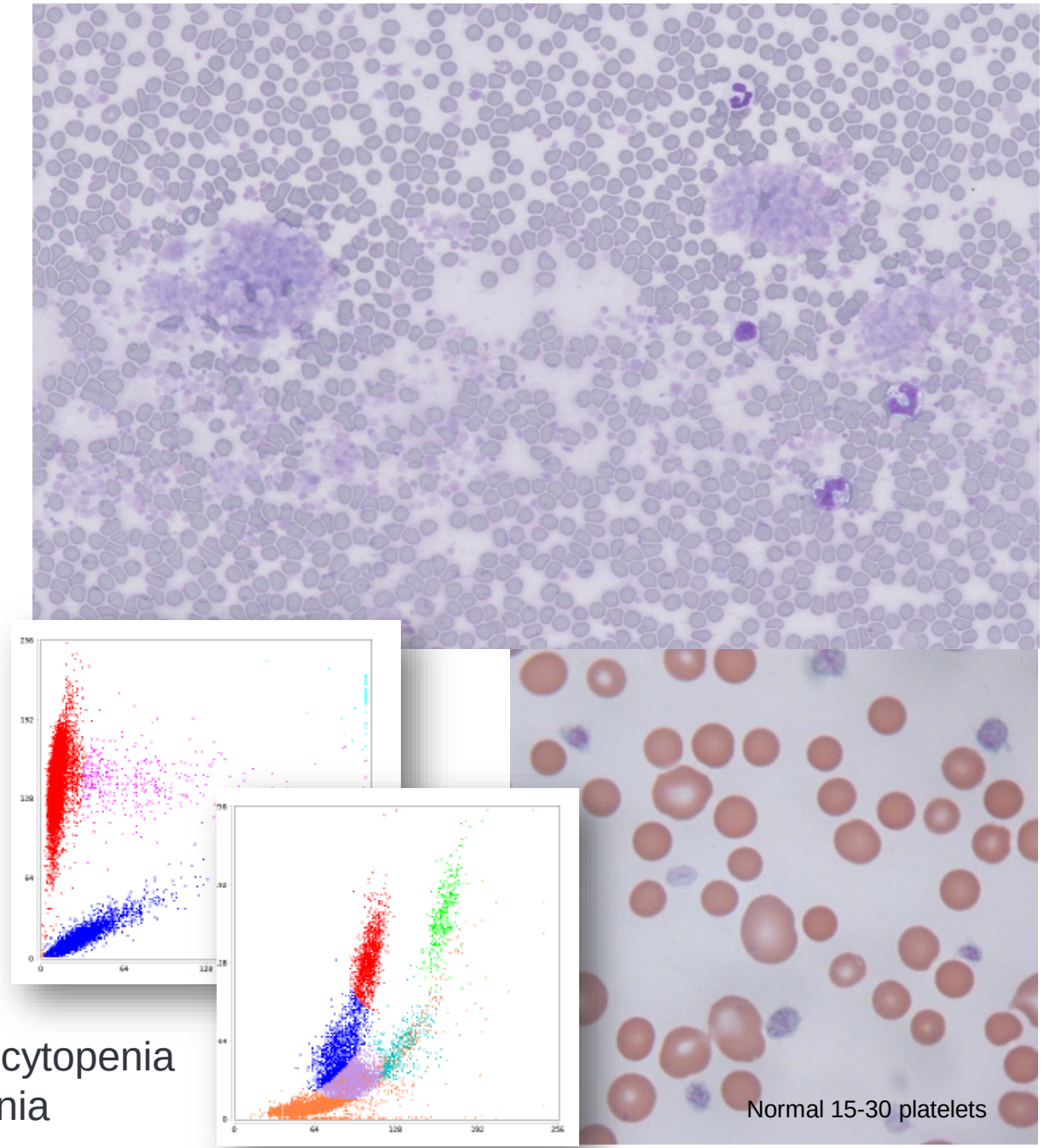
1. Count number of platelets in 10 fields oil immersion in monolayer

2. Do the average (Σ /10)

3. Multiply by 15 or 20

4. This is the ***estimated*** number

   (x 10^9/L)



Normal 15-30 platelets

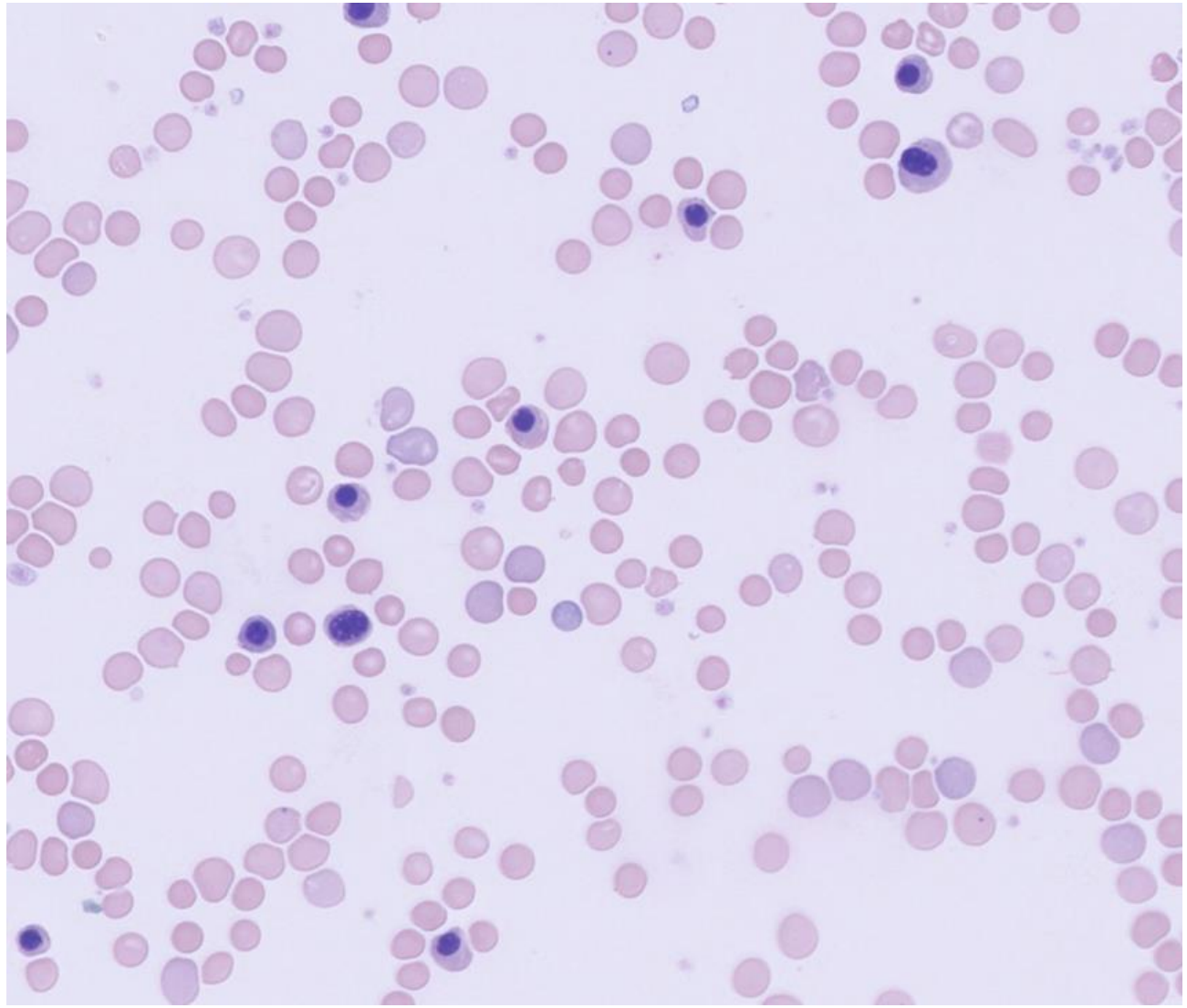
>10 per HPF = no significant thrombocytopenia
<2 per HPF = marked thrombocytopenia

For the RBC we are evaluating 3 main things…

+ Density and spread
    + Agglutination

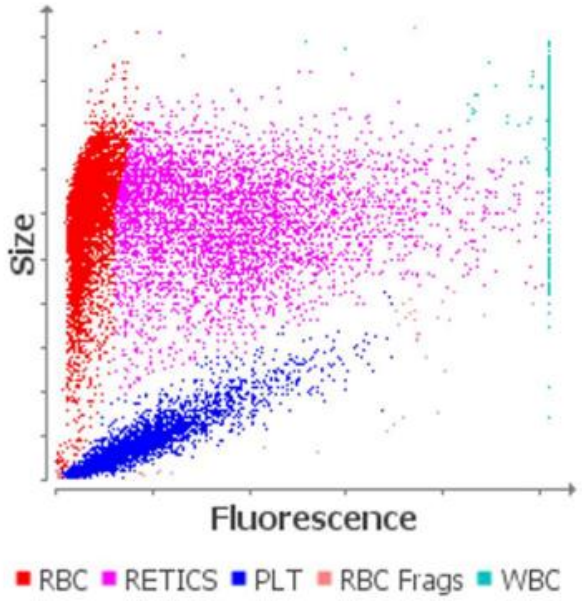
+ Colour
    + Polychromasia
    + Hypochromasia

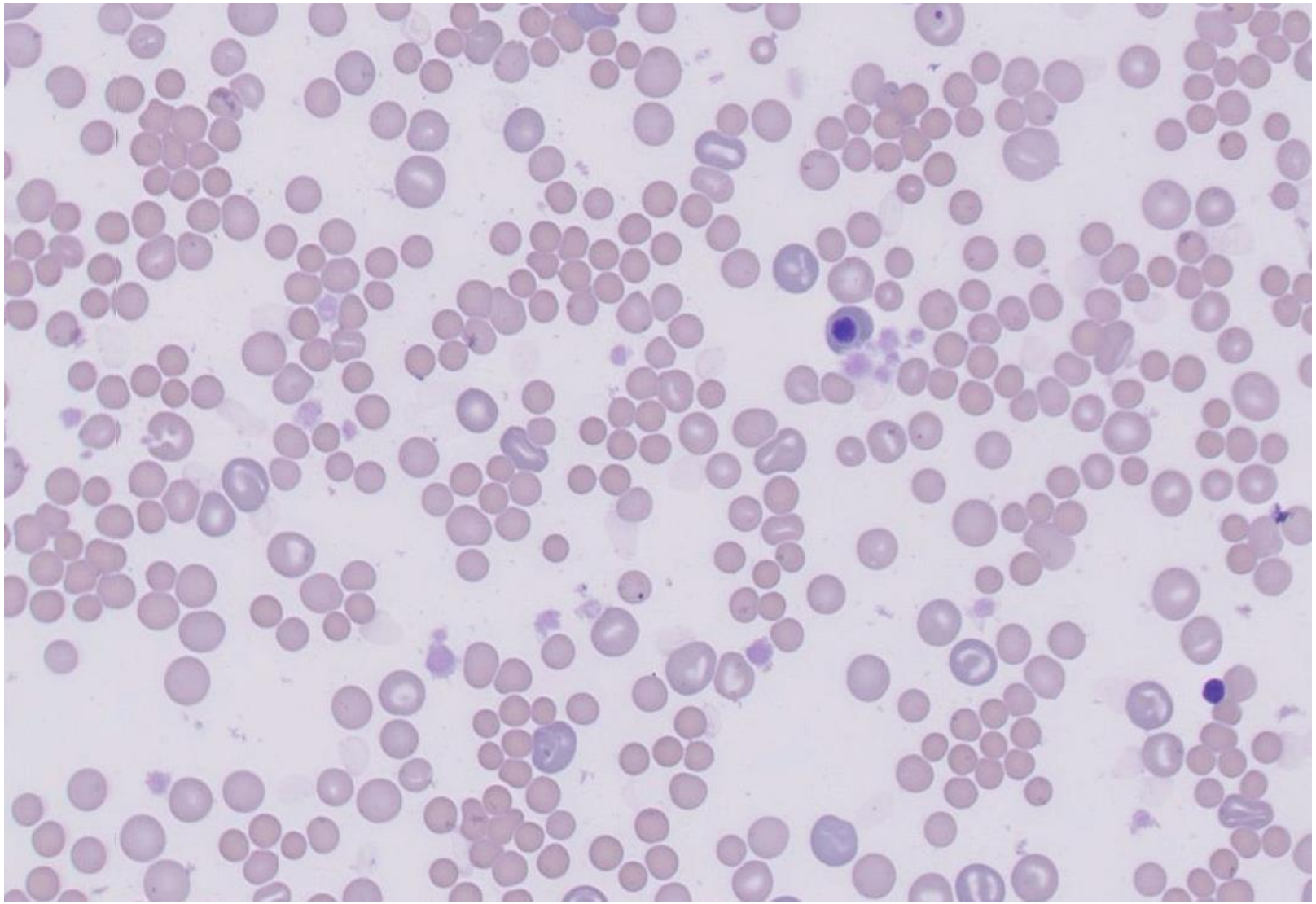
+ Size, shapes and inclusions
    + nRBC
    + Heinz bodies
    + Spherocytes
    + Poikilocytes



Red blood cell abnormalities

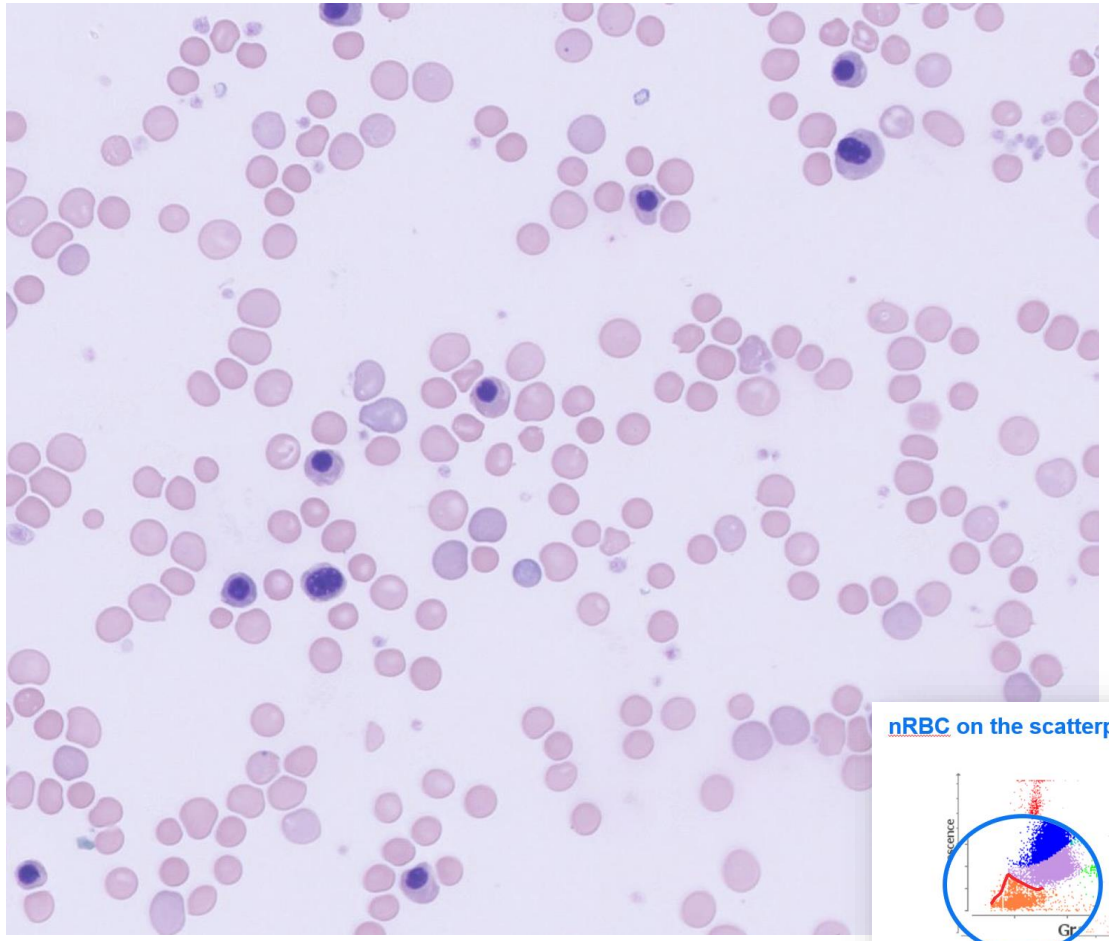
+ Polychromasia



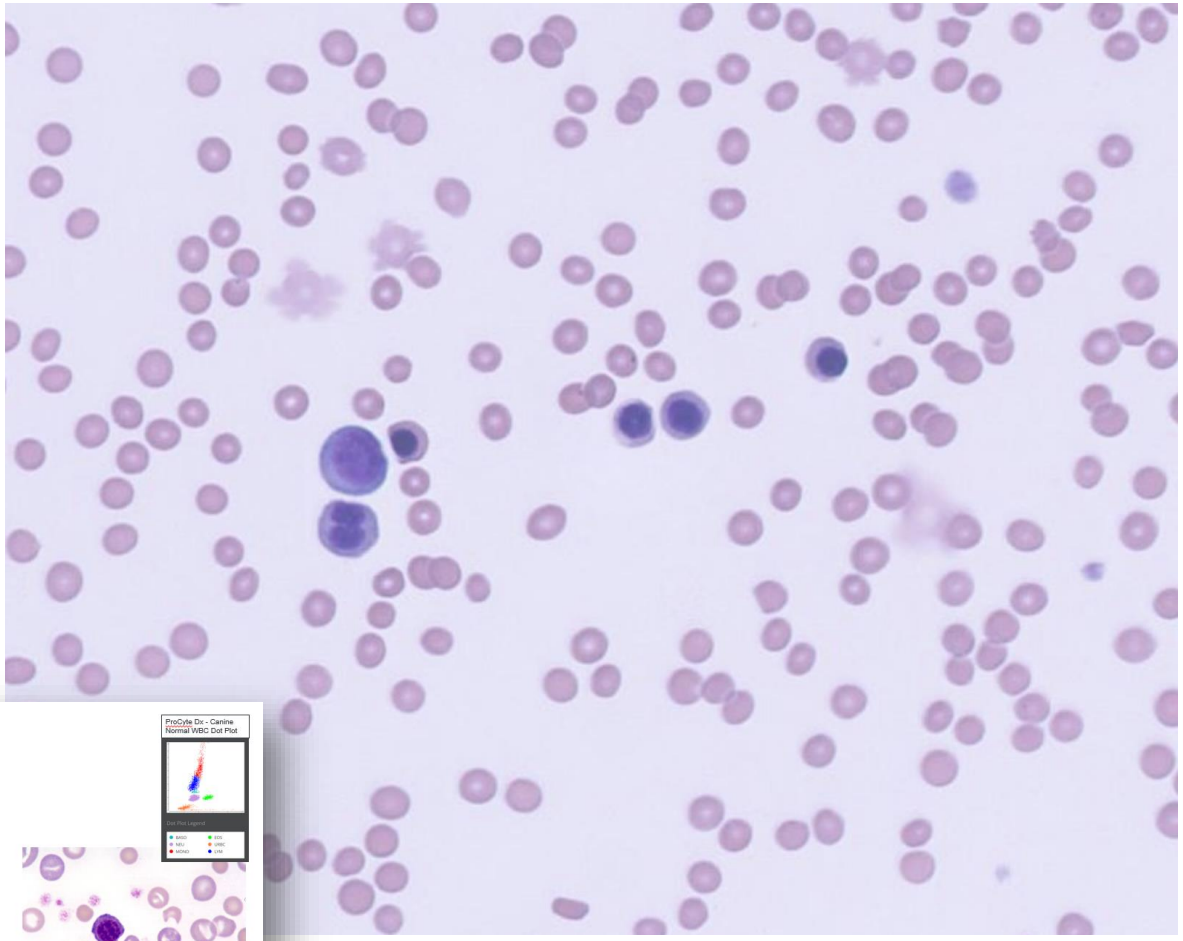


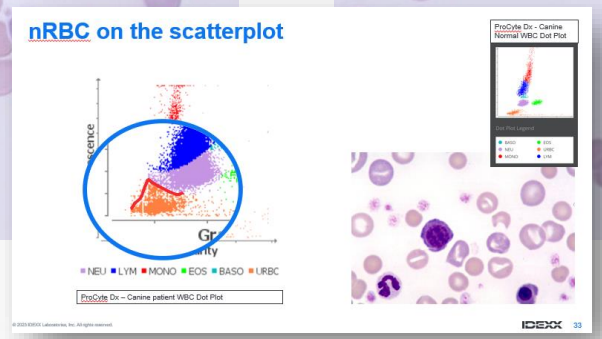
Nucleated RBC

As part of regeneration

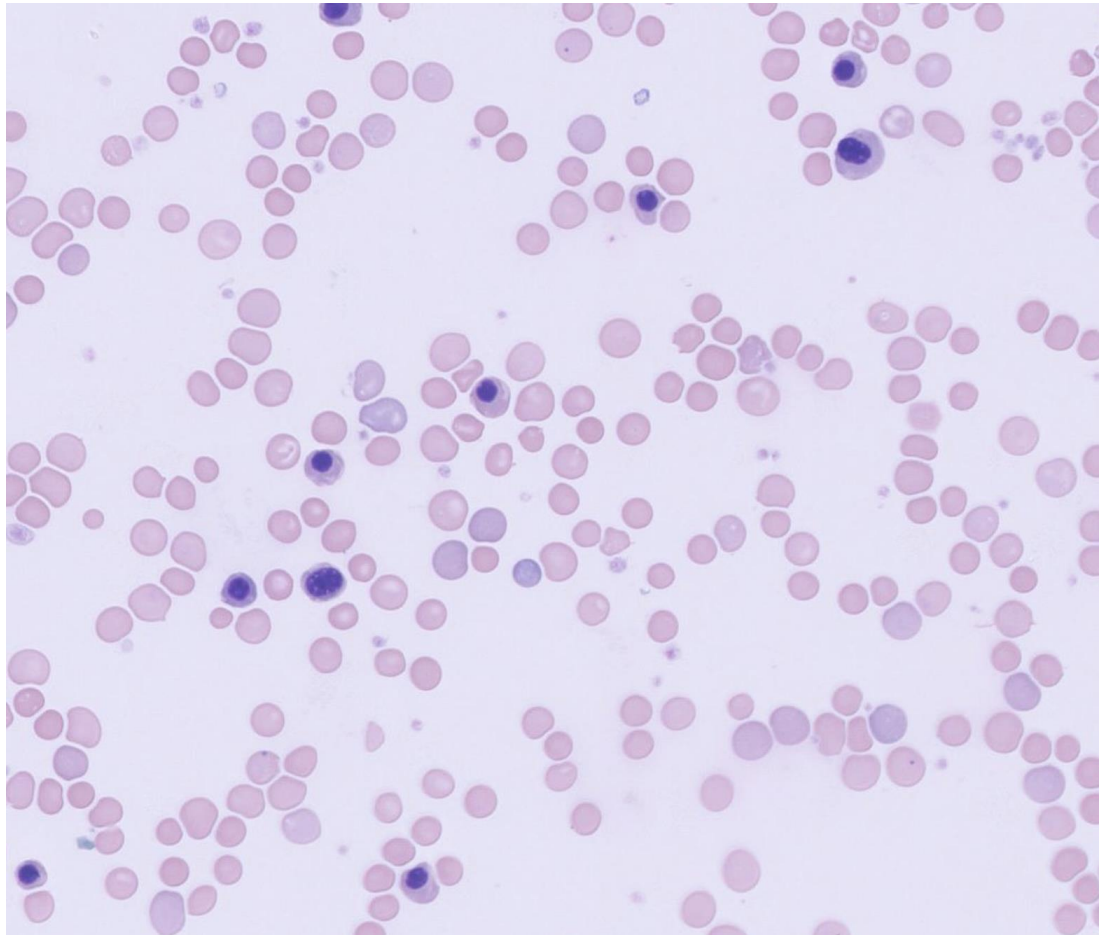


Inappropriate rubricytosis





Nucleated red blood cells



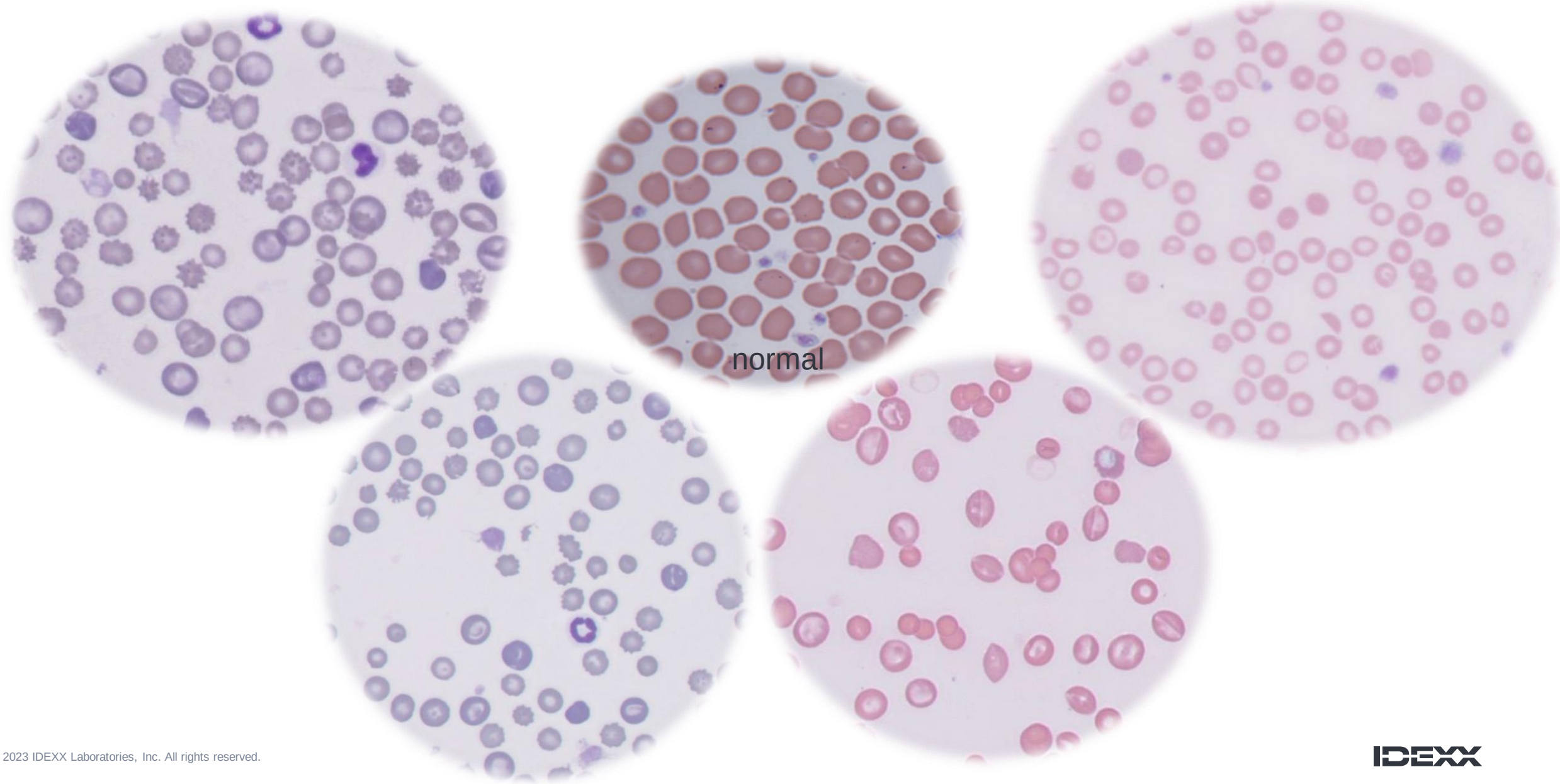
- Part of regenerative response
- Number should be proportionate

- In absence of proportionate regeneration:

  INAPPROPRIATE RUBRICYTOSIS

  - Bone marrow damage
  - Lead poisoning
  - Heat stroke
  - Dyserythropoiesis
  - Splenic disorder

Are there different sizes, shapes or inclusions?



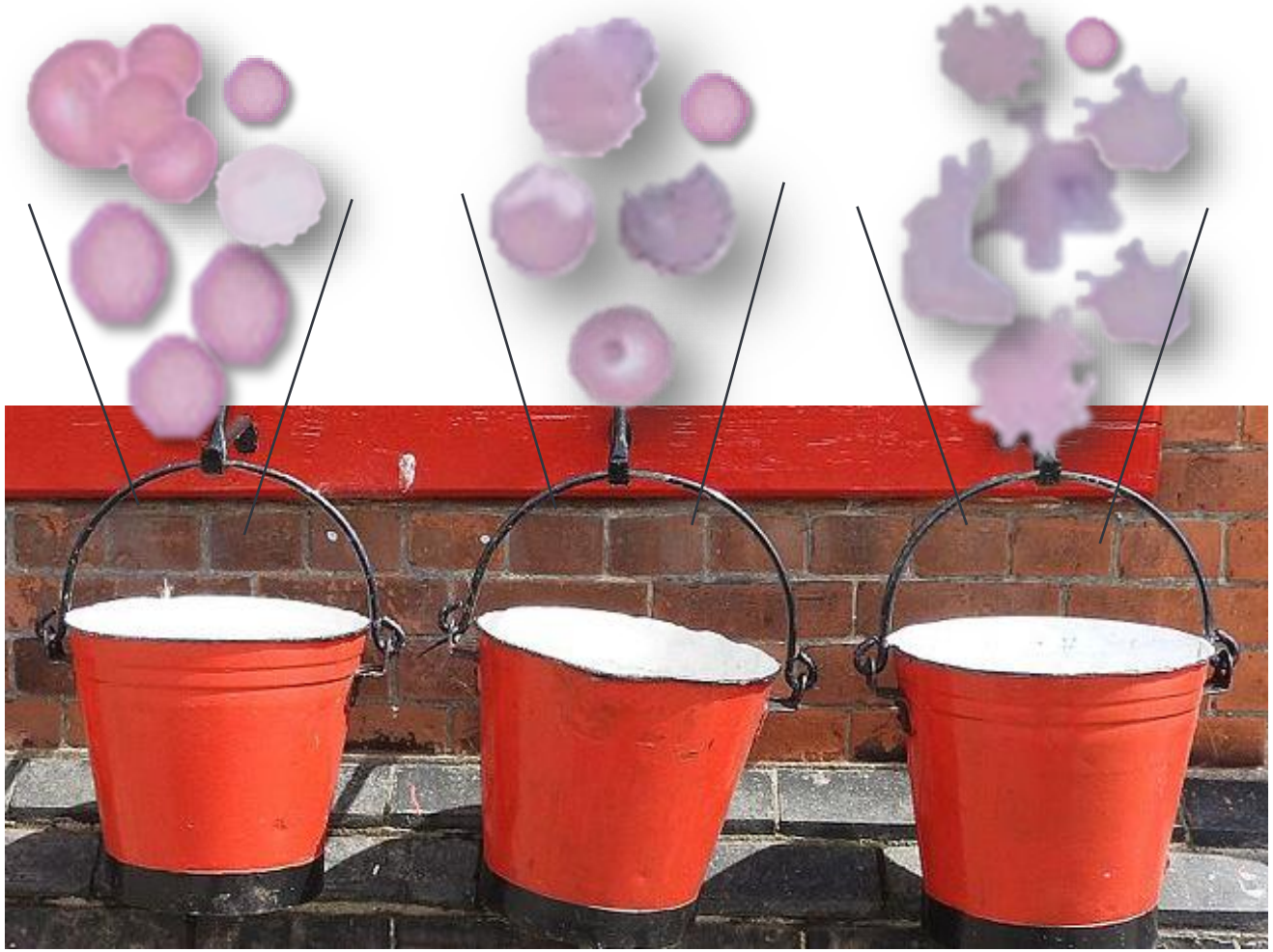
Poikilocytosis (erythrocytes shape changes)

The secret coding language of haematology

+ Spherocytes
+ Ghost cells
+ Acanthocytes
+ Keratocytes
+ Schistocytes
+ Heinz bodies
+ Echinocytes (crenated RBC)
+ Torocytes
+ Codocytes/target cells
+ Eccentrocytes
+ Ovalocytes
+ Dacreocytes
+ Knizocytes
+ Leptocytes

And the list goes on…

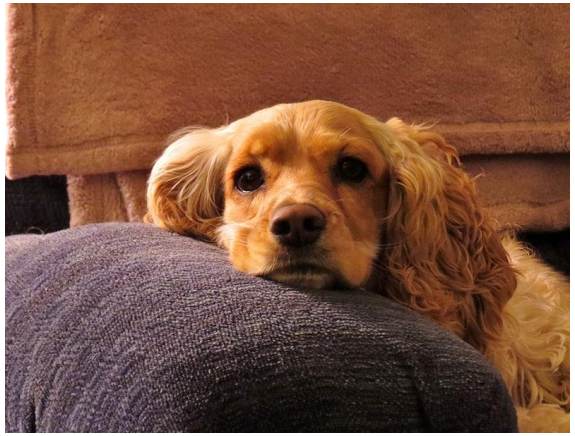
Poikilocytosis (erythrocytes shape changes)

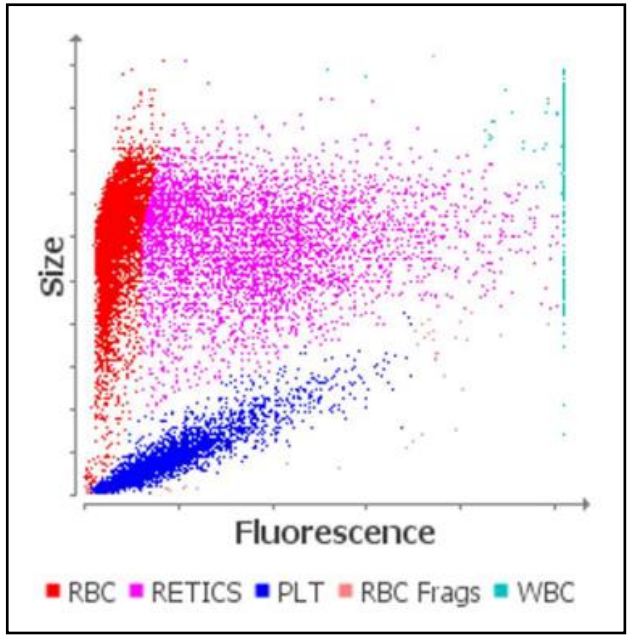


Two young anaemic dogs

<u>Case 1</u> Molly 4 yo FN Cocker Spaniel

- Lethargic for the past few days
- Off food
- Presented collapsed
- HR 140 and tachypnoea
- PCV 14.8%



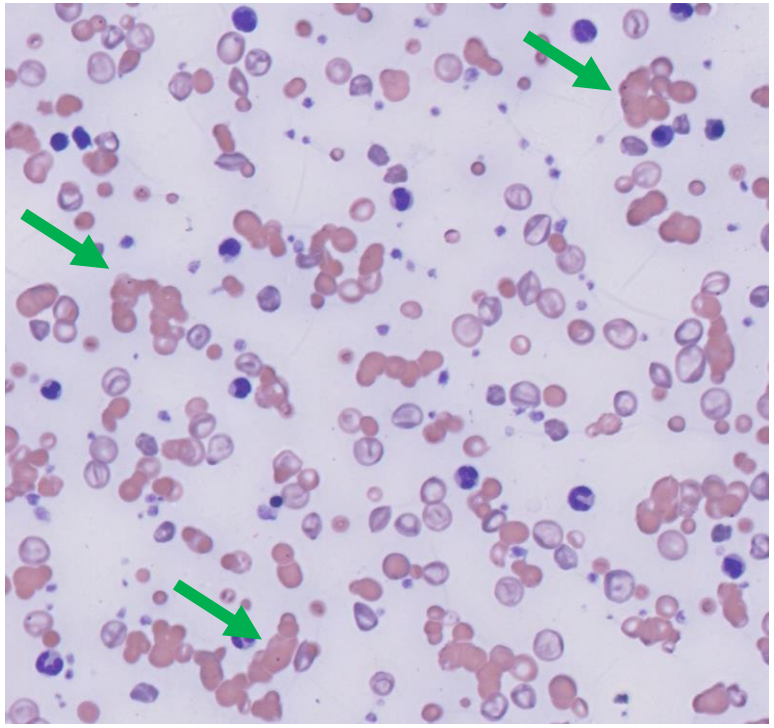


<u>Case 2</u> Dodger 2 yo ME Border Collie

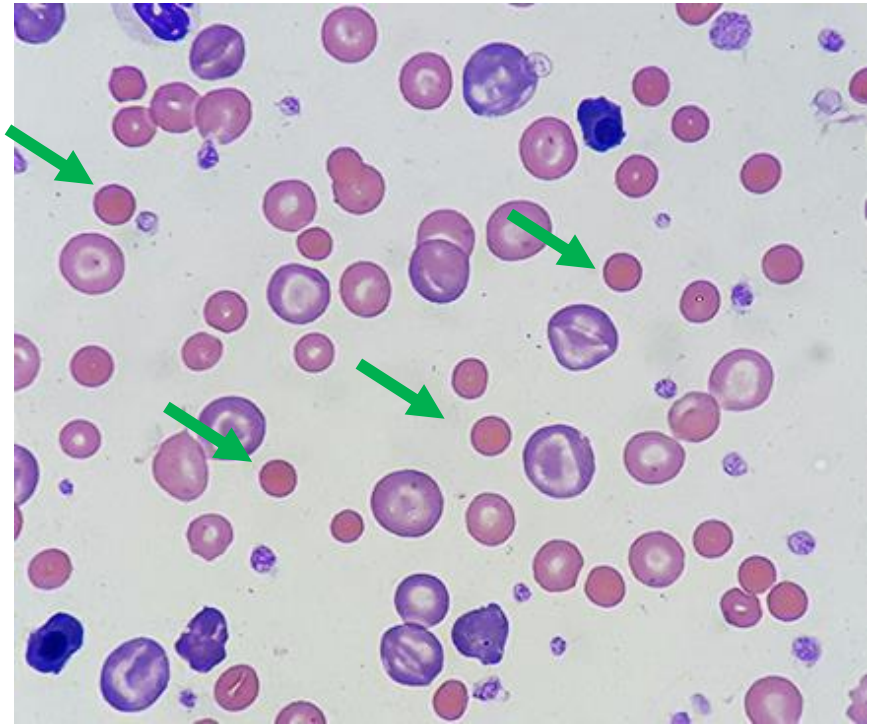
- Very quiet for a couple of days
- Off food
- Mucous membranes very pale
- HR 160
- PCV 20.1%

Case 1 - Molly

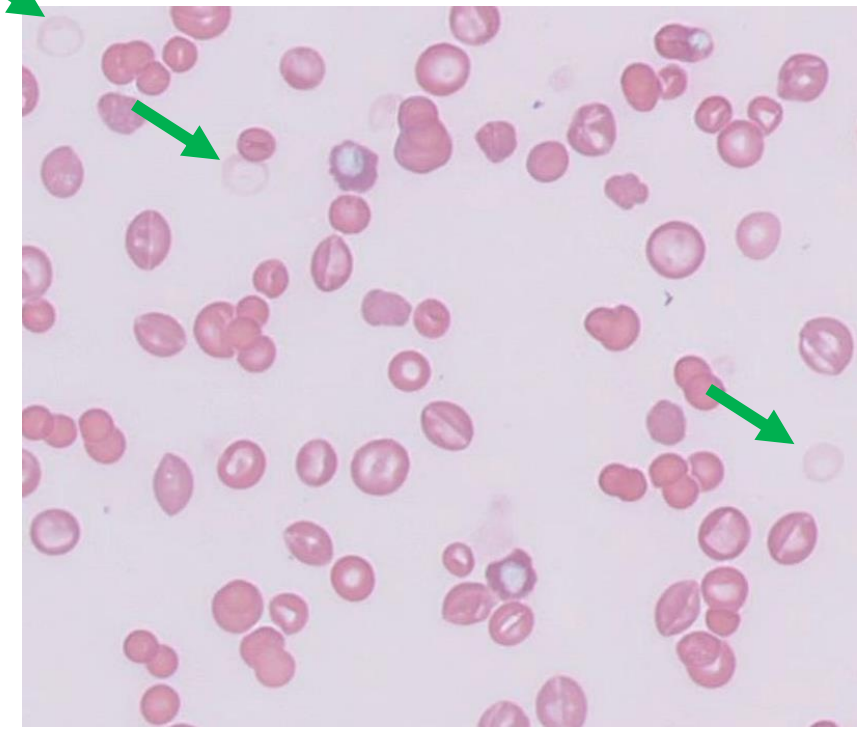
Features suggestive of **immune mediated haemolytic anaemia**



Agglutination
(Saline agglutination test)
(+/- Coombs test)



Spherocytes



Ghost cells (fresh smear)

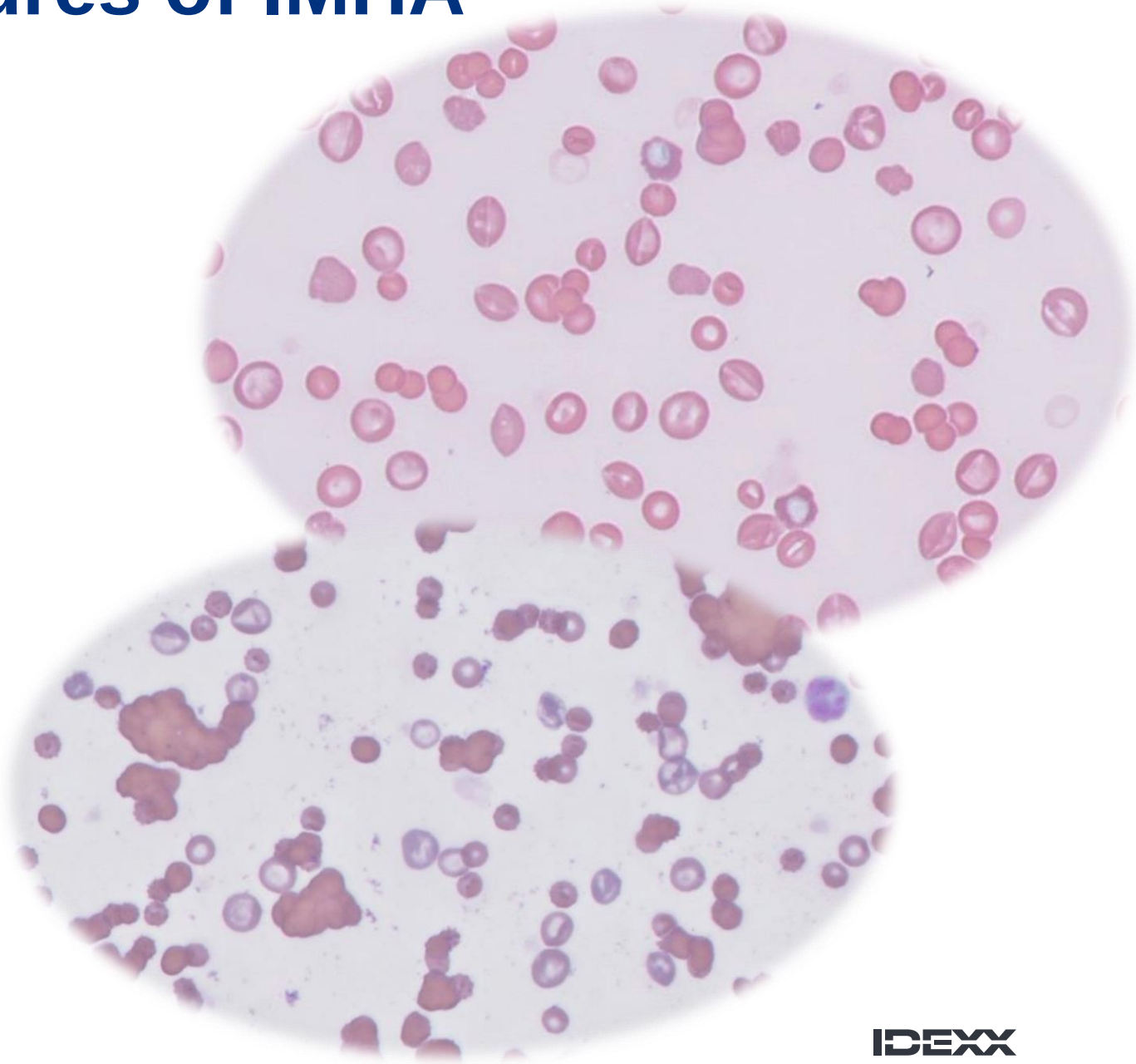
RBC morphology: Features of IMHA

- Agglutination
- Spherocytosis (Dogs)

- Ghost cells
- Ideally on a fresh smear

- Questionable results/follow up tests
  - In-saline agglutination
  - +/- Coomb’s test

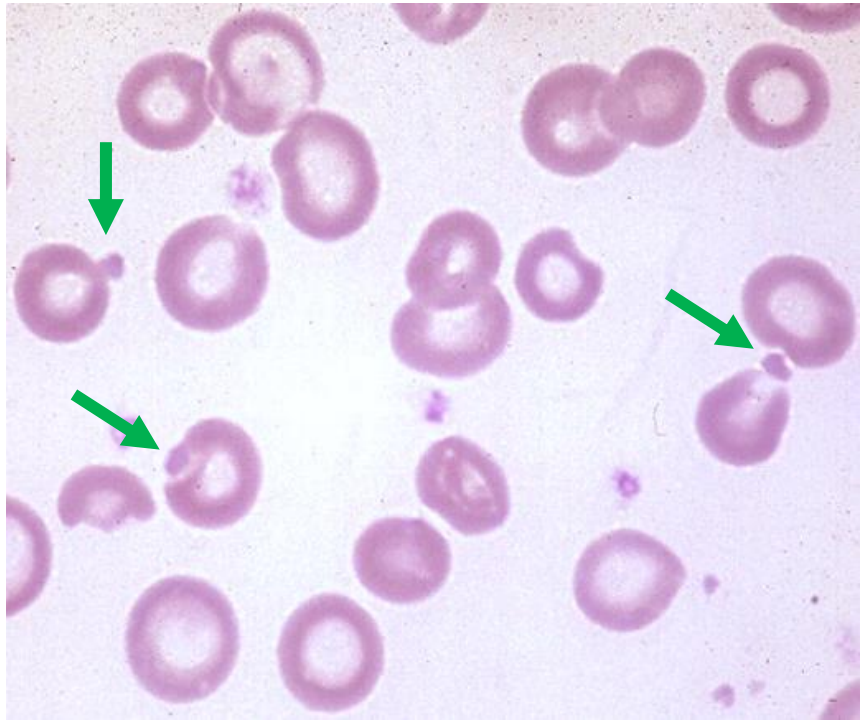
- Regenerative vs Non-regenerative



IDEXX

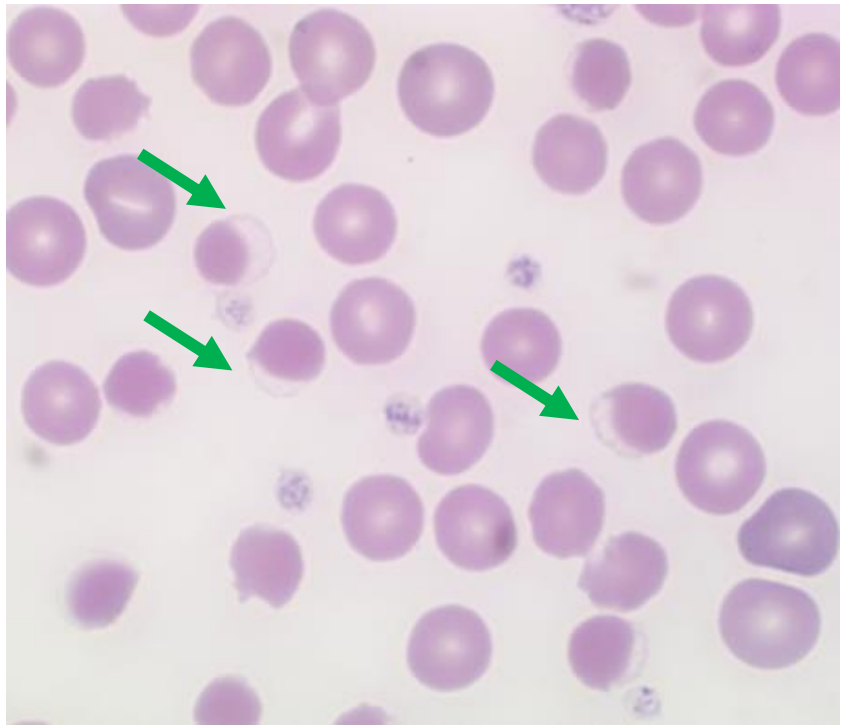
Case 2 - Dodger

Features suggestive of
haemolytic anaemia due to oxidative injury



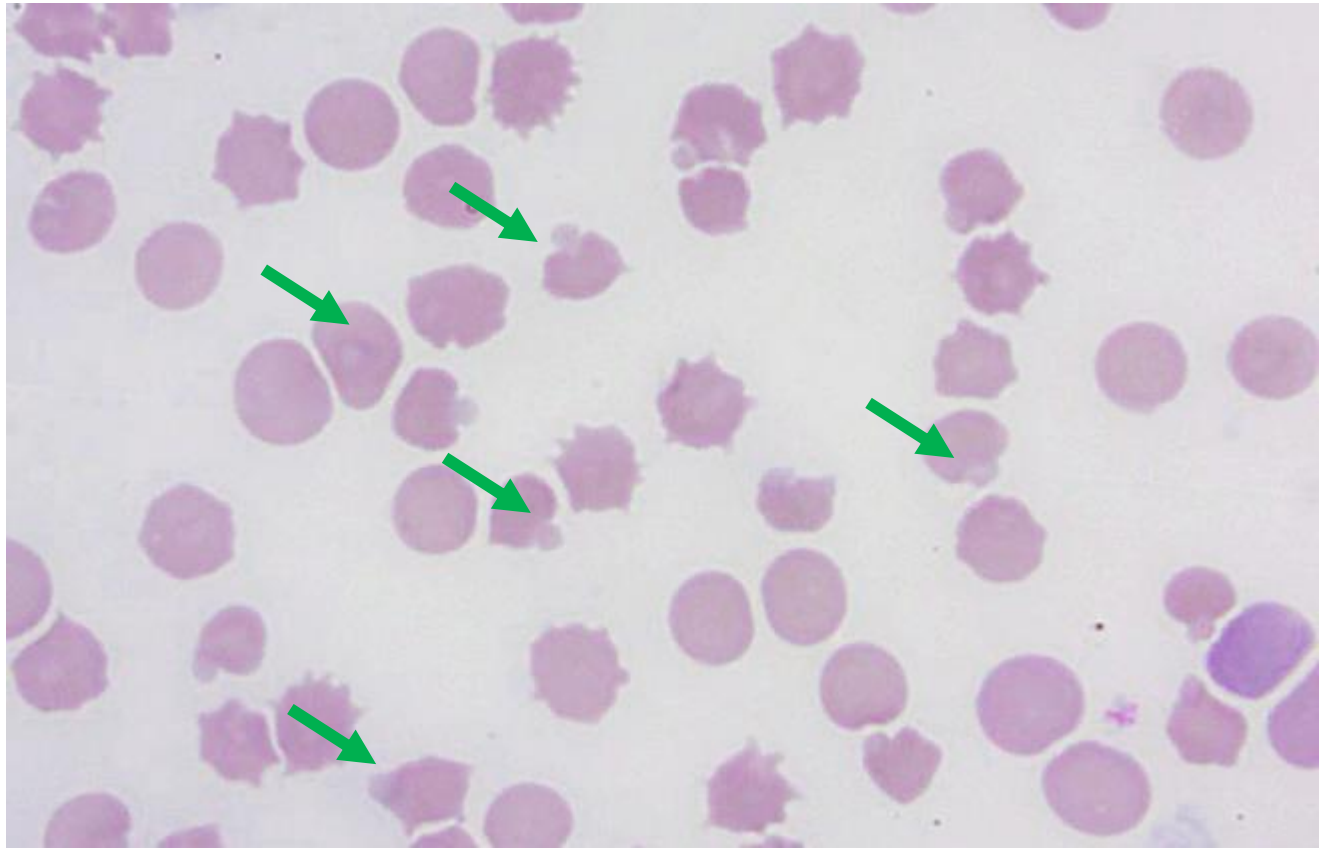
Heinz bodies

Heinz body on ghost cell

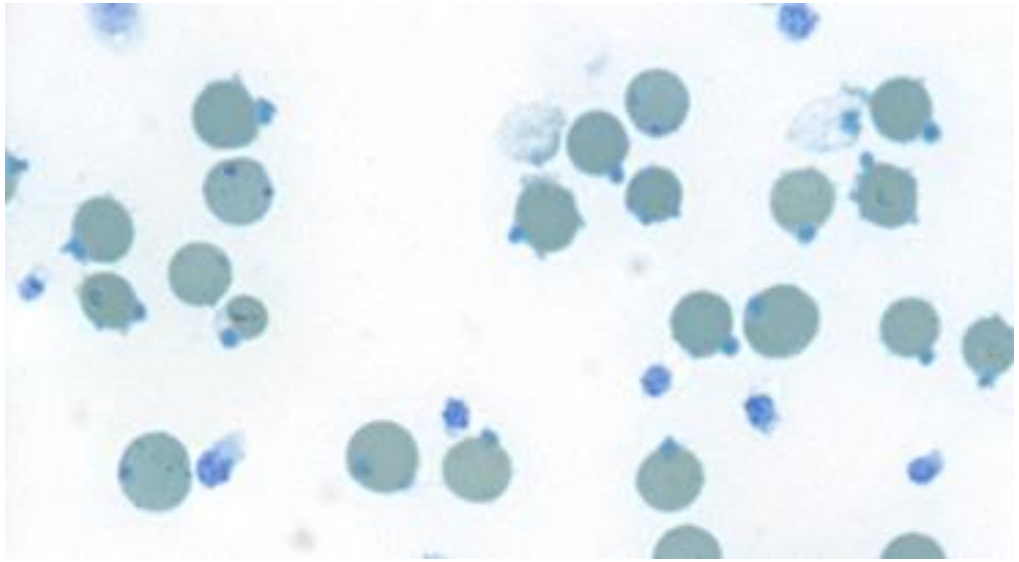


Eccentrocytes

Heinz bodies in cats



- Small numbers normal in cats
- When increased often due to endogenous oxidants
- inflammation
- diabetes mellitus (ketoacidosis)
- hyperthyroidism
- neoplasia (lymphoma)

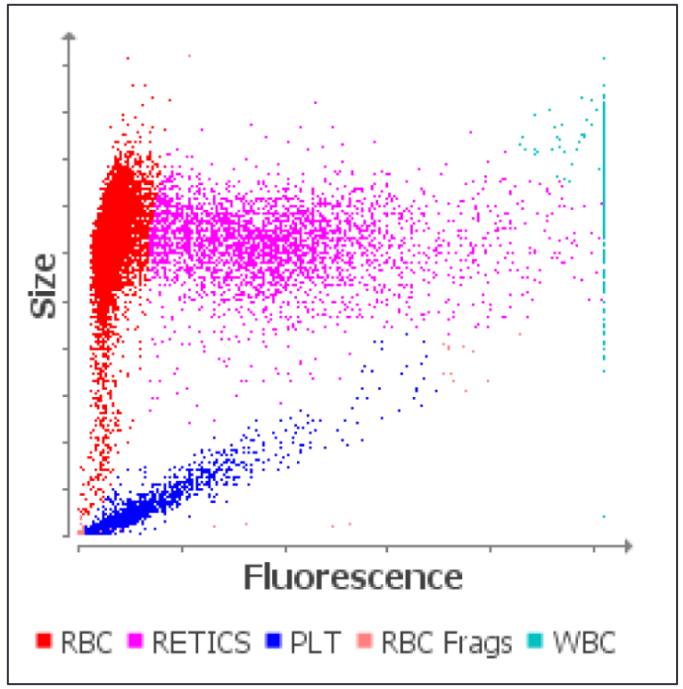


And the case of an old anaemic dog

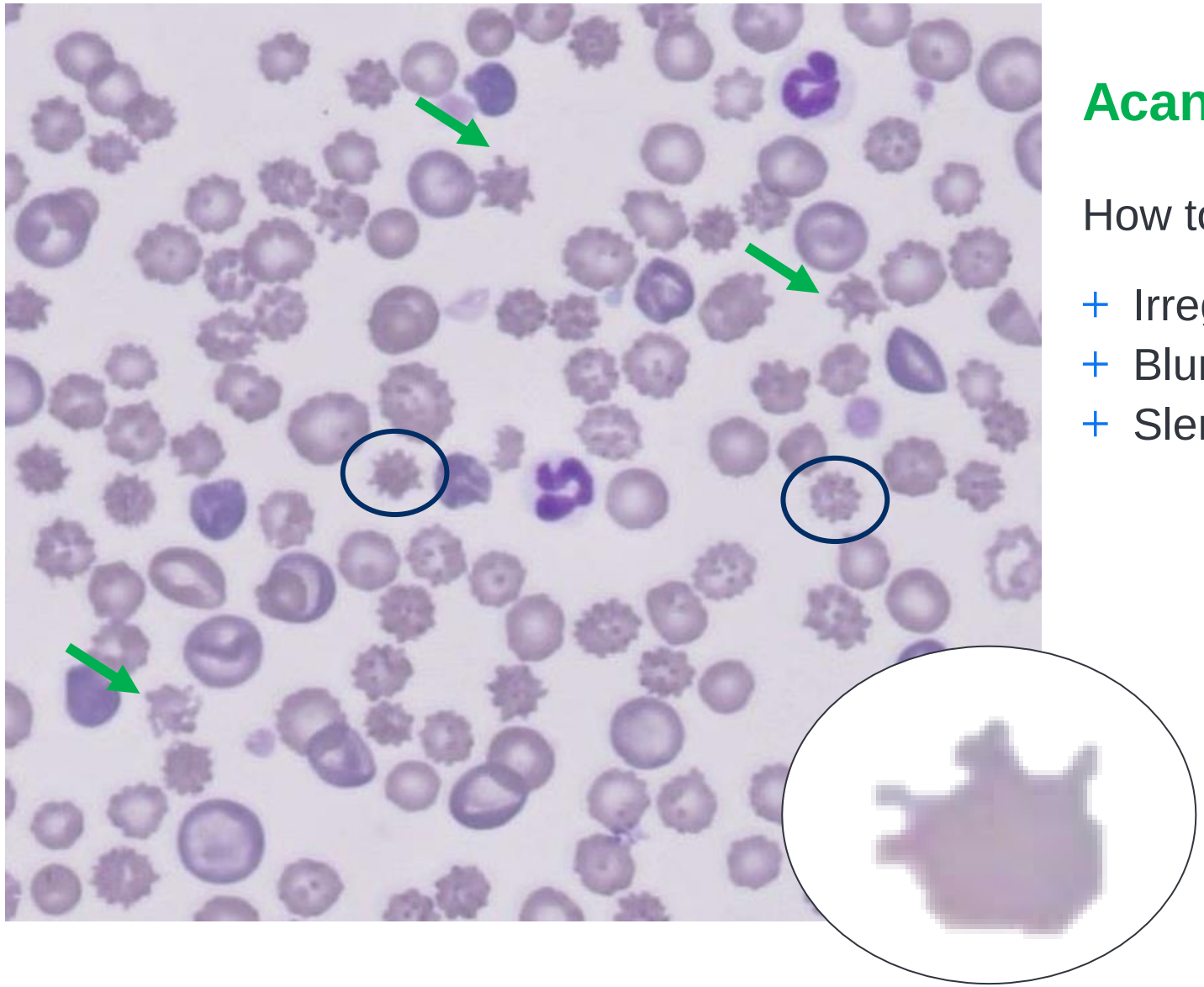
Case 3 Buster 12 yo MN Mix breed dog

+ Occasional vomiting recently
+ Presented collapsed
+ Tachypnoea and tachycardia
+ Distended abdomen
+ Pale mucus membranes

+ PCV 19.4%



Case 3 - Buster

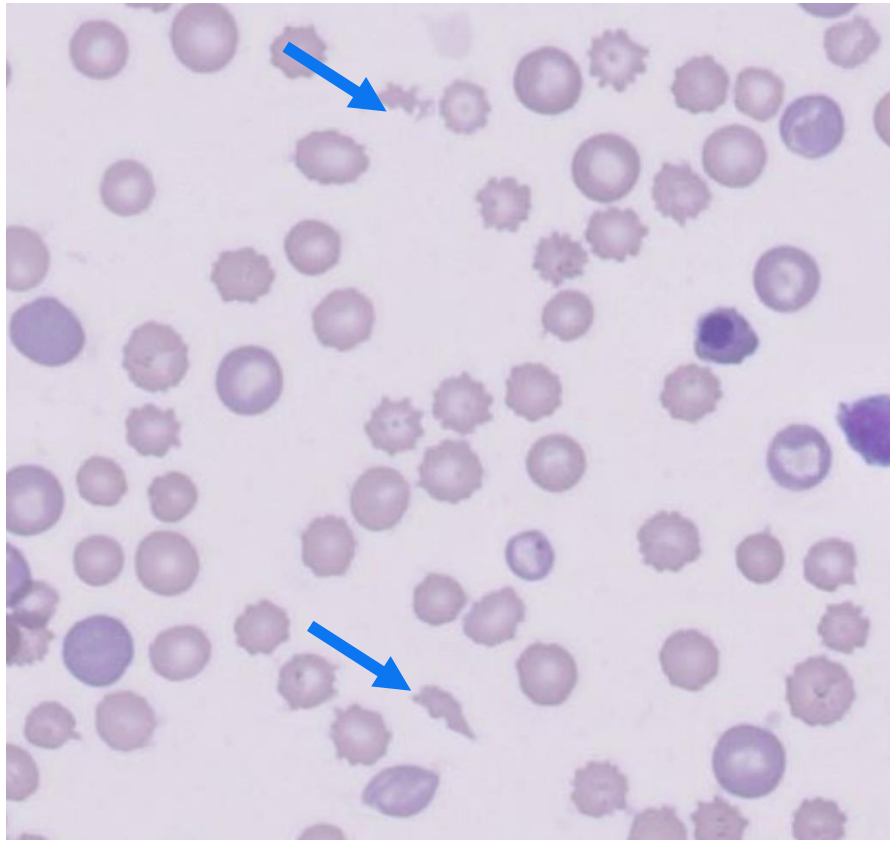


Acanthocytes

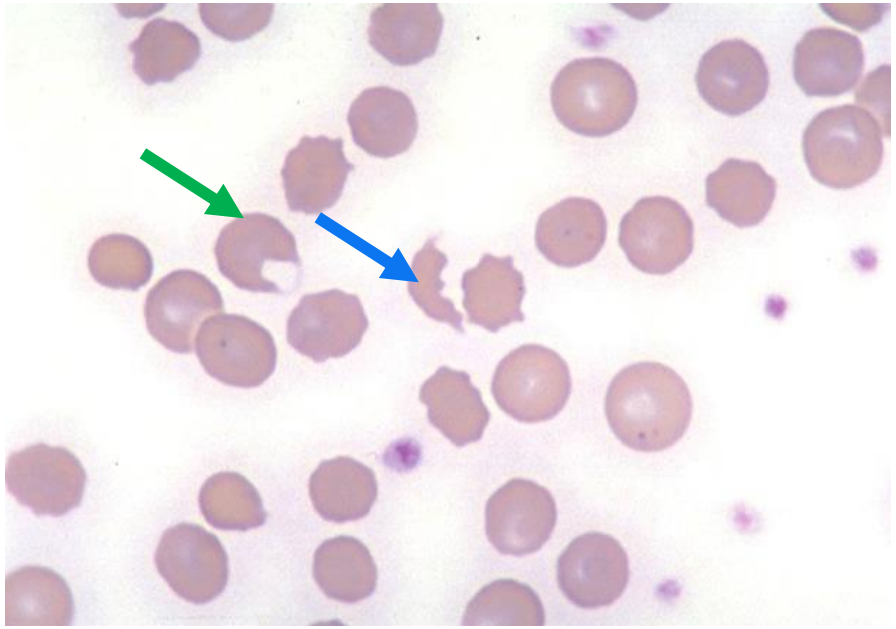
How to differentiate from echinocyte (artefact)

+ Irregular, unevenly distributed
+ Blunt-ended
+ Slender, long thread-like projections

Case 3 - Buster



Schistocytes



Keratocytes

Features suggestive of
RBC fragmentation (microangiopathy)

Poikilocytes

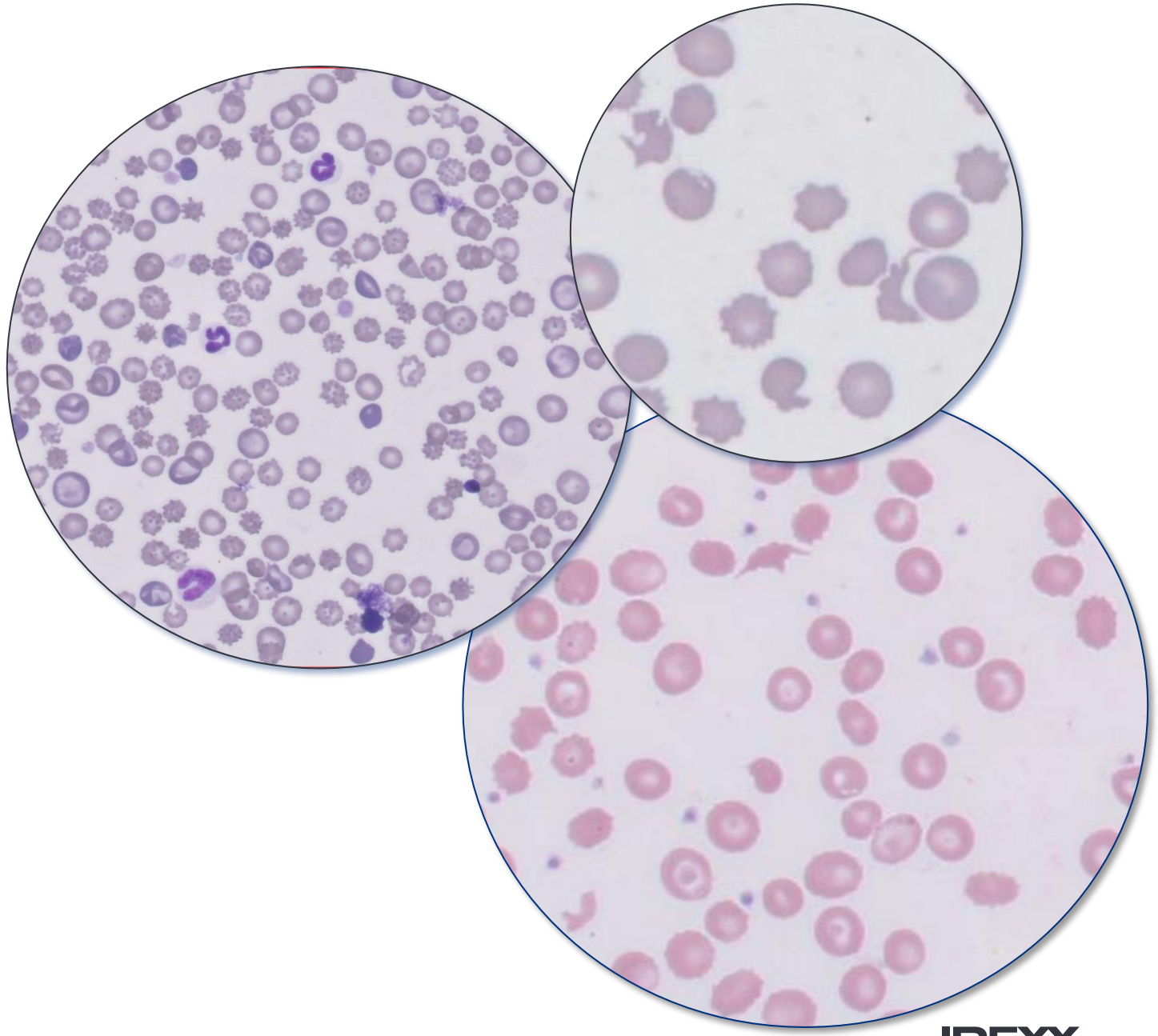
Significance?

- >1/2 per 100X field?
- Associated anaemia?
- Polychromasia?

Common differentials:

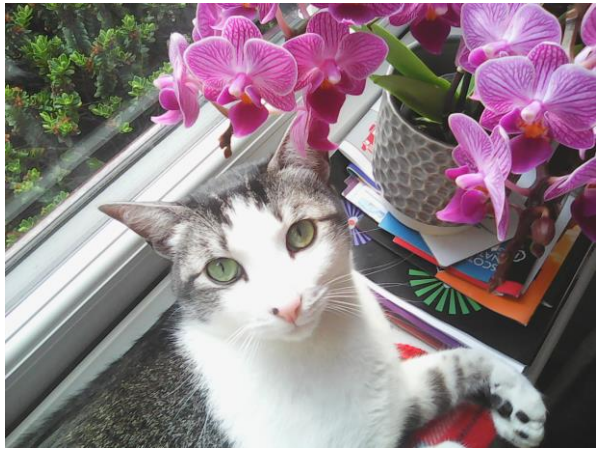
Conditions causing vascular abnormalities or turbulent blood flow, e.g.:

- Vascular tumours (haemangiosarcoma)
- Splenic/liver disease
- DIC
- Vasculitis
- Glomerulonephritis



White blood cell abnormalities

When the numbers don’t tell everything

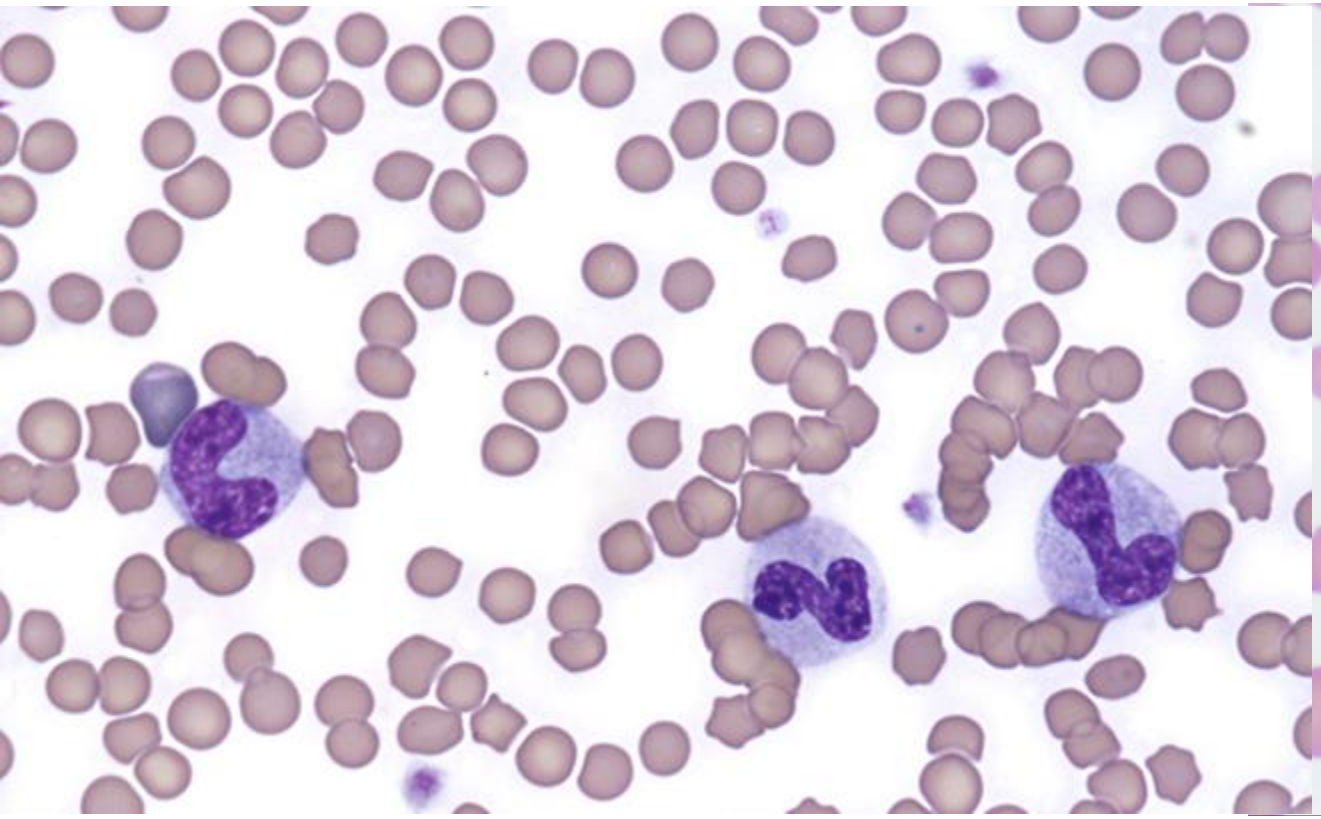


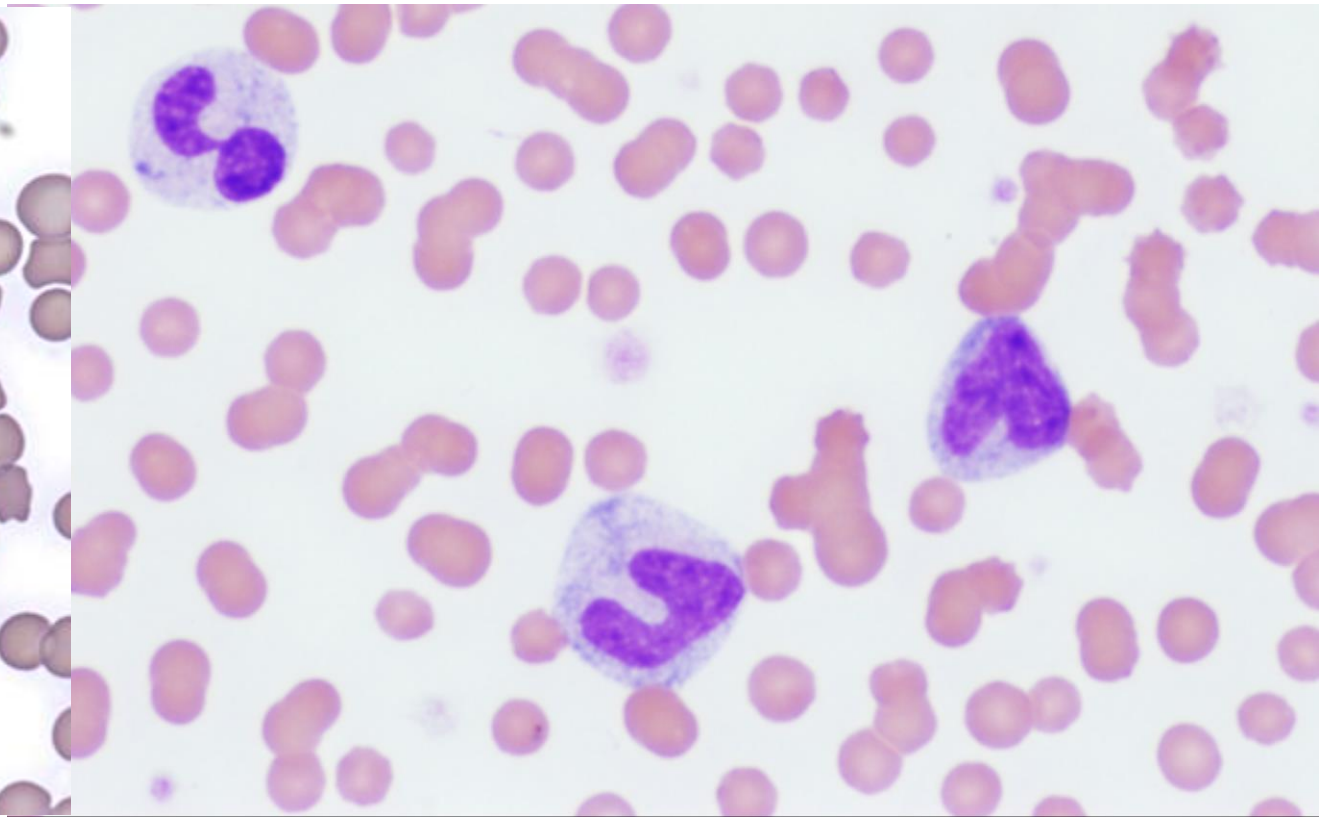
Case 4 Bella 8yo FN Domestic Shorthair

+ Lethargic
+ Anorexia
+ Occasional vomiting

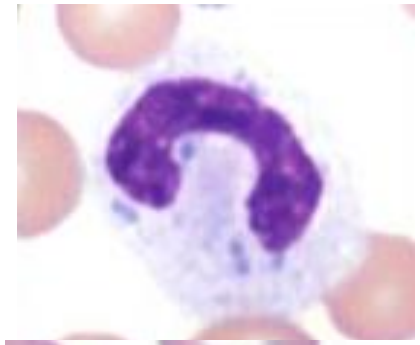
WBC	6.26	2.87 - 17.02 x10^9/L
Neutrophils	4.05	2.30 - 10.29 x10^9/L
Lymphocytes	1.42	0.92 - 6.88 x10^9/L
Monocytes	0.24	0.05 - 0.67 x10^9/L
Eosinophils	0.46	0.17 - 1.57 x10^9/L
Basophils	0.09	0.01 - 0.26 x10^9/L

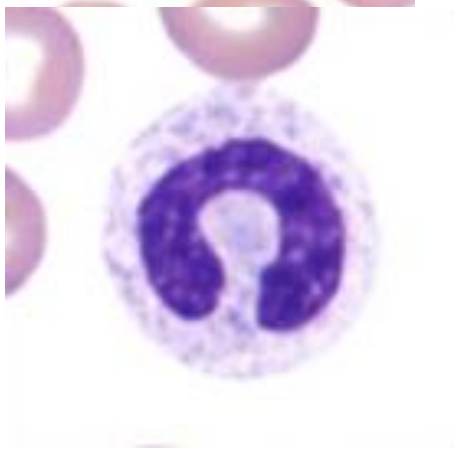
Case 4 - Bella

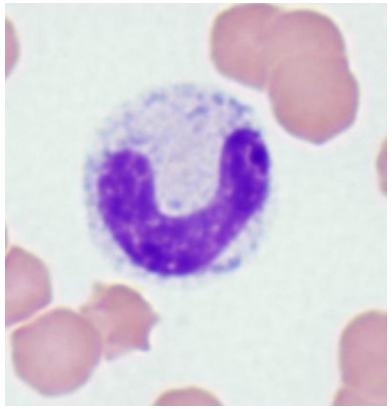




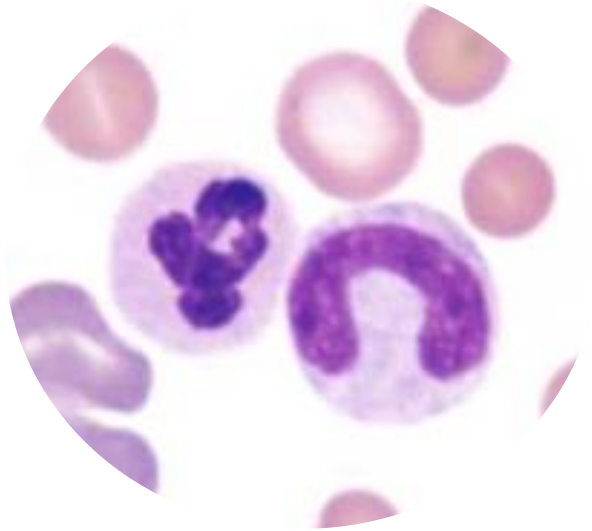
Case 4 - Bella





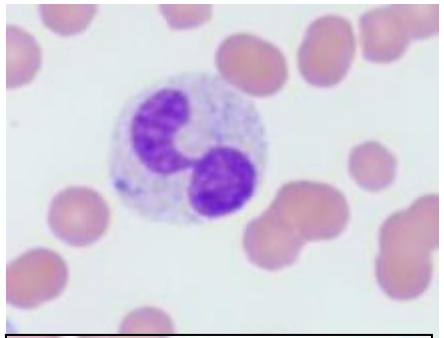


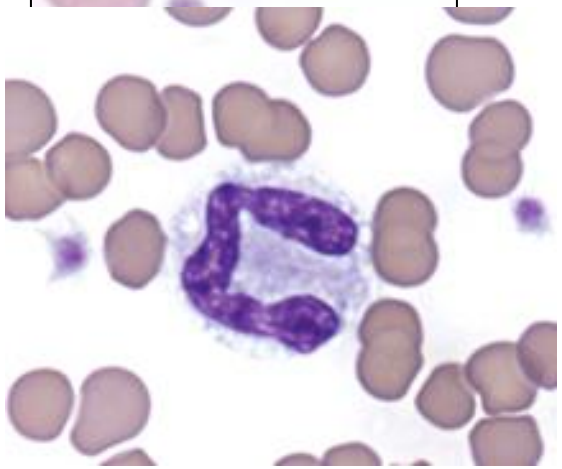
+ Left shift



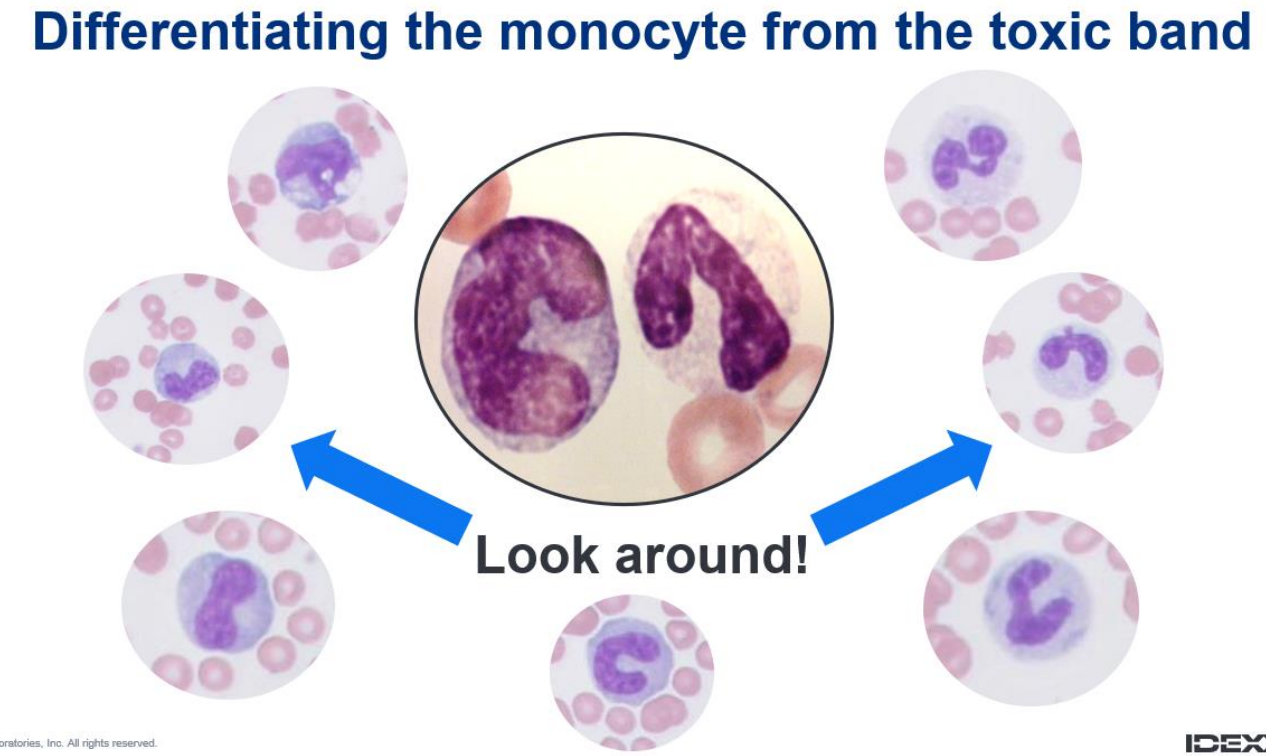
	% Neutrophils	76.0	%
	% Bands	12.0	%
	% Lymphocytes	4.0	%
	% Monocytes	7.0	%
	% Eosinophils	1.0	%
	% Basophils	0.0	%

Case 4 - Bella





- Toxic changes
  - Dohle bodies (*cats)
  - Cytoplasmic basophilia
  - Foamy cytoplasm



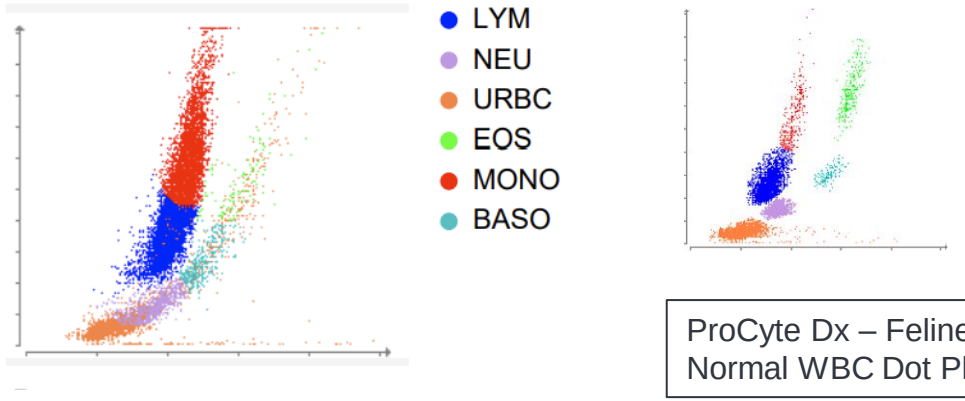
Case 4 - Bella

On further investigations, Bella was diagnosed with a GI foreign body (hairball) with secondary peritonitis

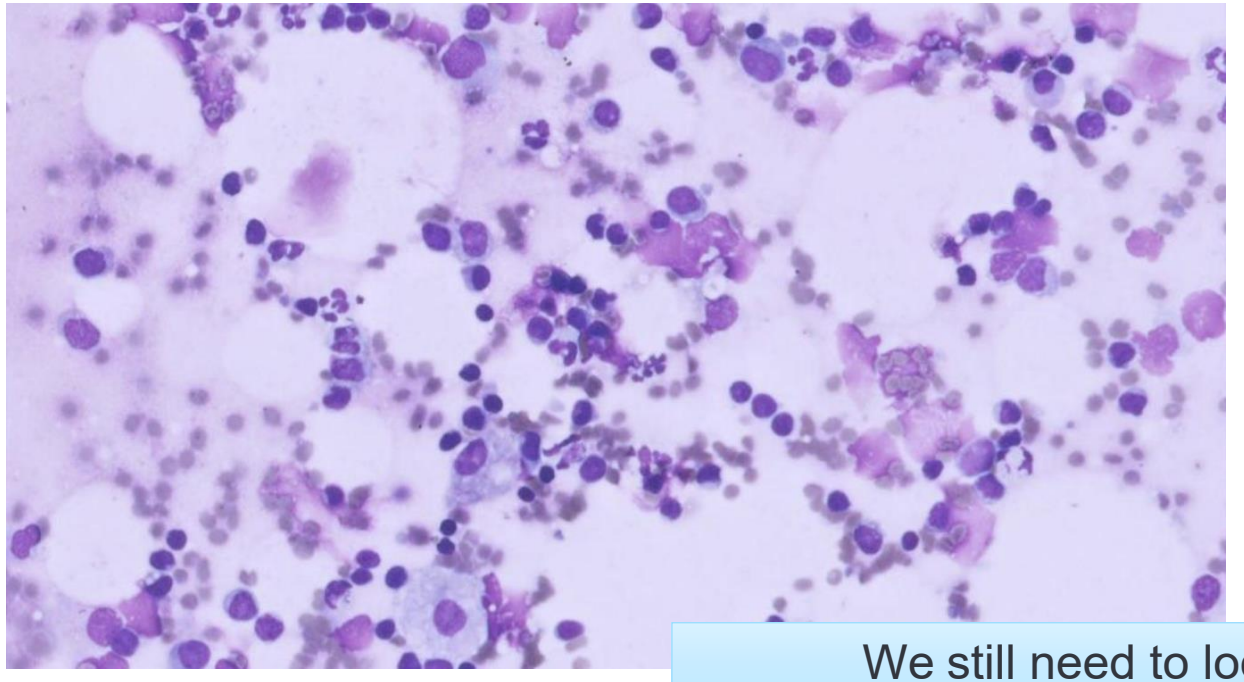
Case 5 - Nefeli

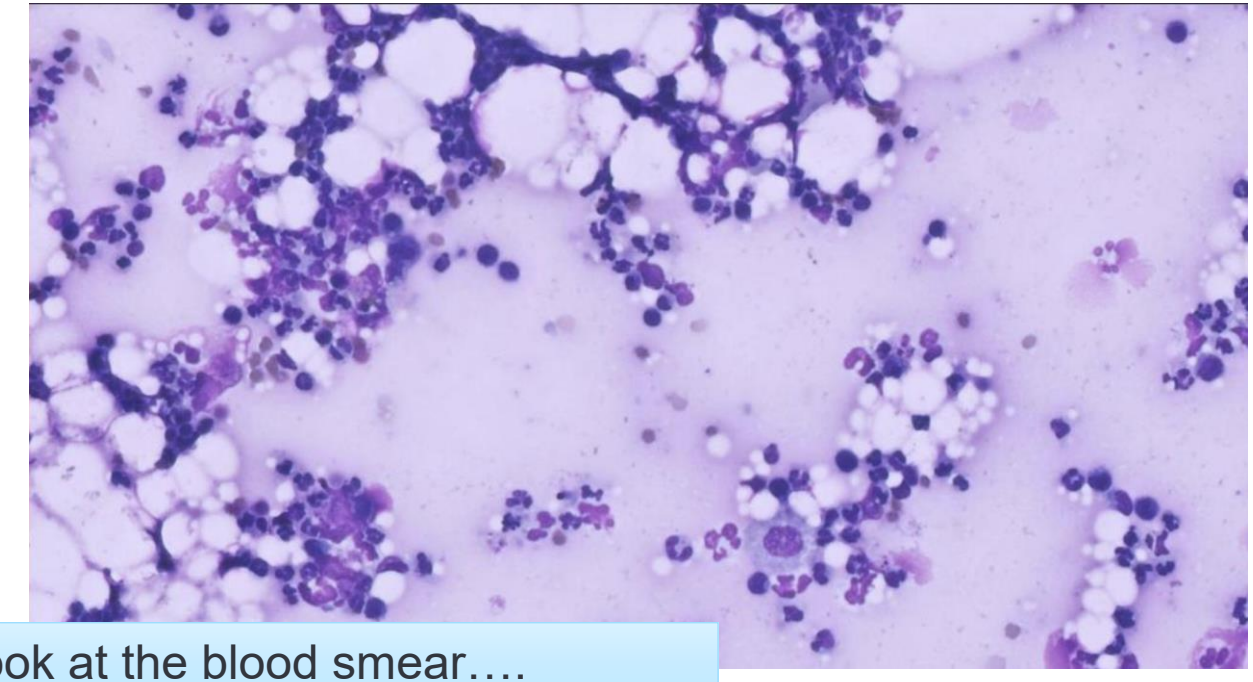
- 6Y FN
- History of lethargy, anorexia, ascites, jaundice/48 hours.
- Abdominal ultrasound - moderate amount of free fluid (chylous?), large mass in mid/caudal abdomen consistent with mesenteric lymph nodes, liver diffusely enlarged and hyperechoic.
- Ascitic fluid submitted for analysis.
- 1st site - liver, 2nd site - abdominal mass (lymph node)

WBC	*17.78	2.87 - 17.02 x10^9/L	
% Neutrophils	*8.3	%	
% Lymphocytes	*46.2	%	
% Monocytes	*42.7	%	
% Eosinophils	*0.7	%	
% Basophils	- --.--	%	
Neutrophils	*1.47	2.30 - 10.29 x10^9/L	
Bands	*Suspected		
Lymphocytes	*8.21	0.92 - 6.88 x10^9/L	
Monocytes	*7.60	0.05 - 0.67 x10^9/L	
Eosinophils	*0.12	0.17 - 1.57 x10^9/L	
Basophils	- --.--	0.01 - 0.26 x10^9/L	

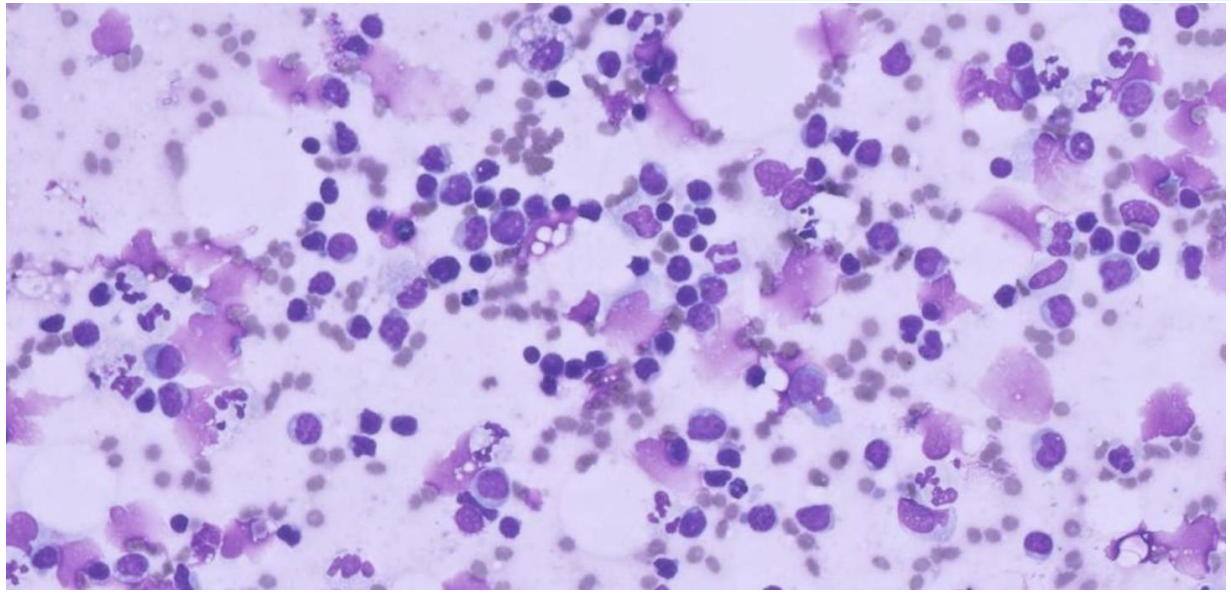


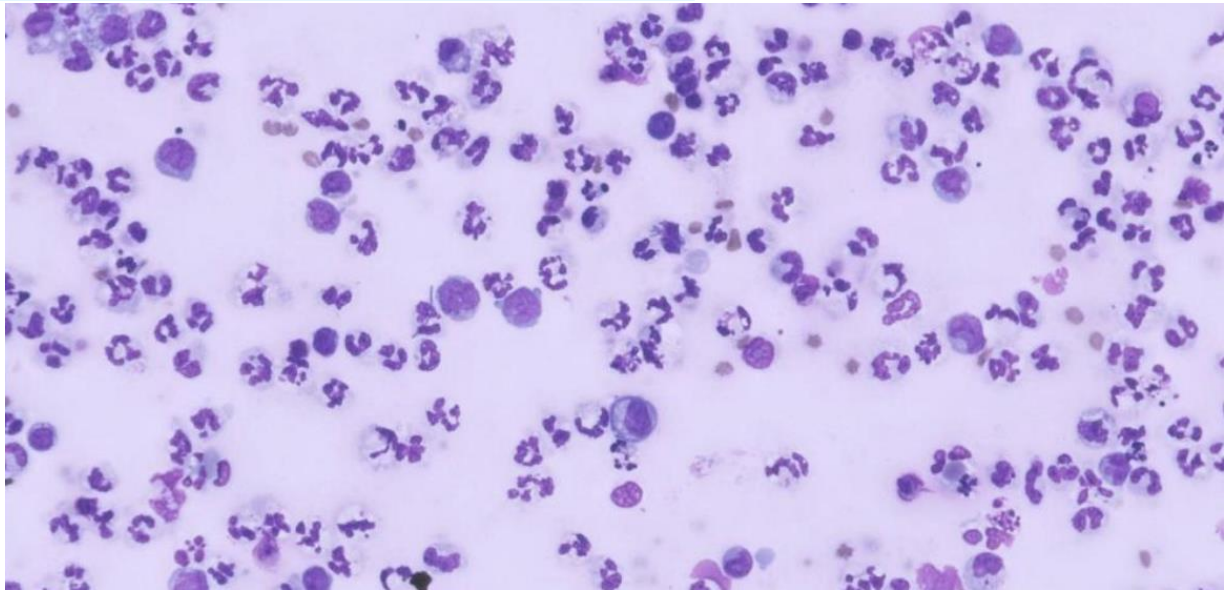
ProCyte Dx – Feline
Normal WBC Dot Plot



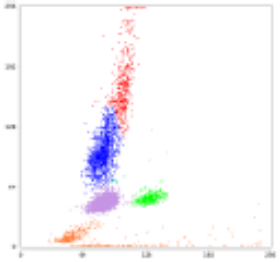


We still need to look at the blood smear….

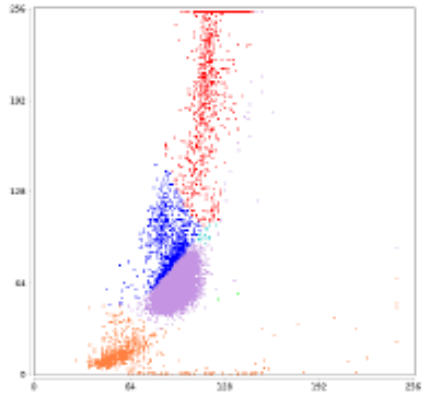


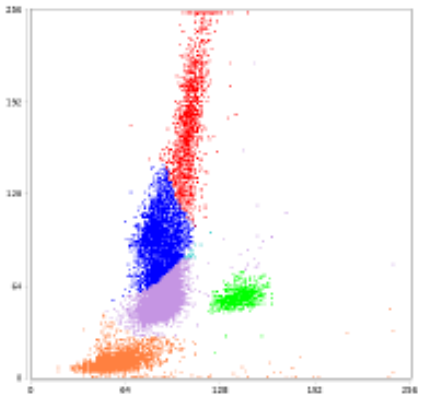


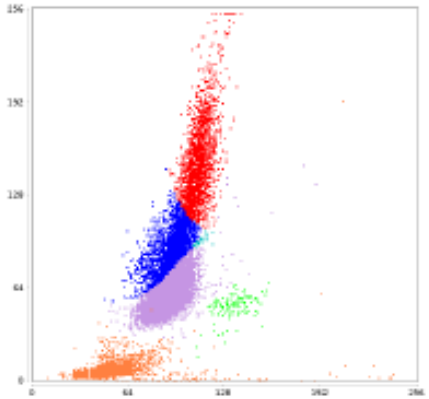
ProCyte DX – examples of left shift

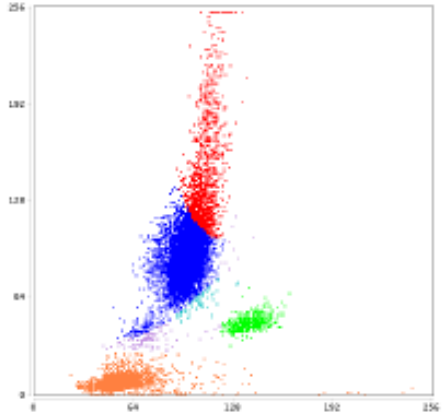


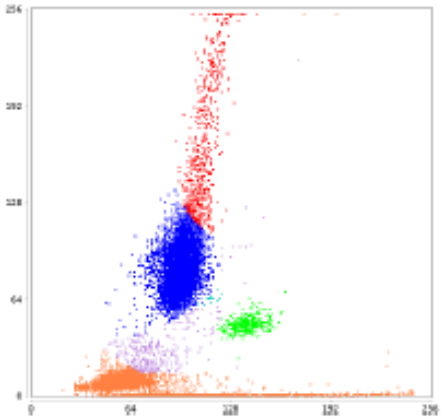
Normal

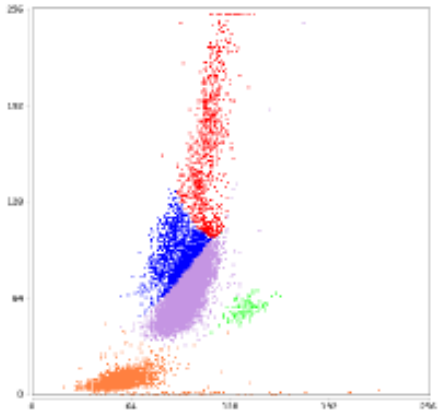


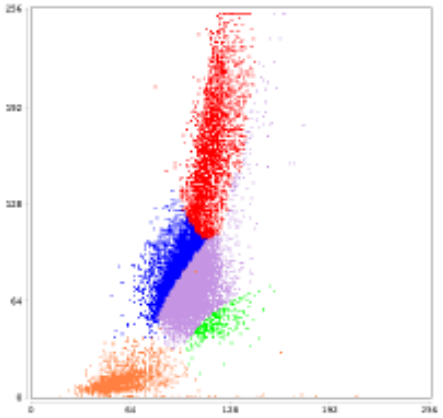


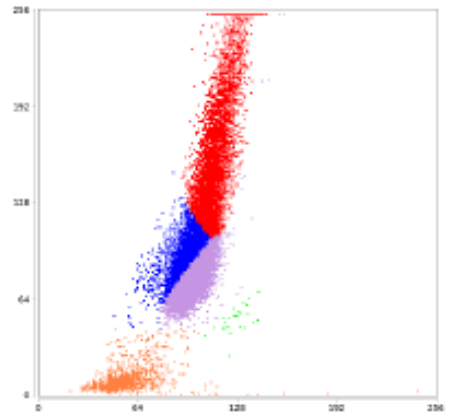




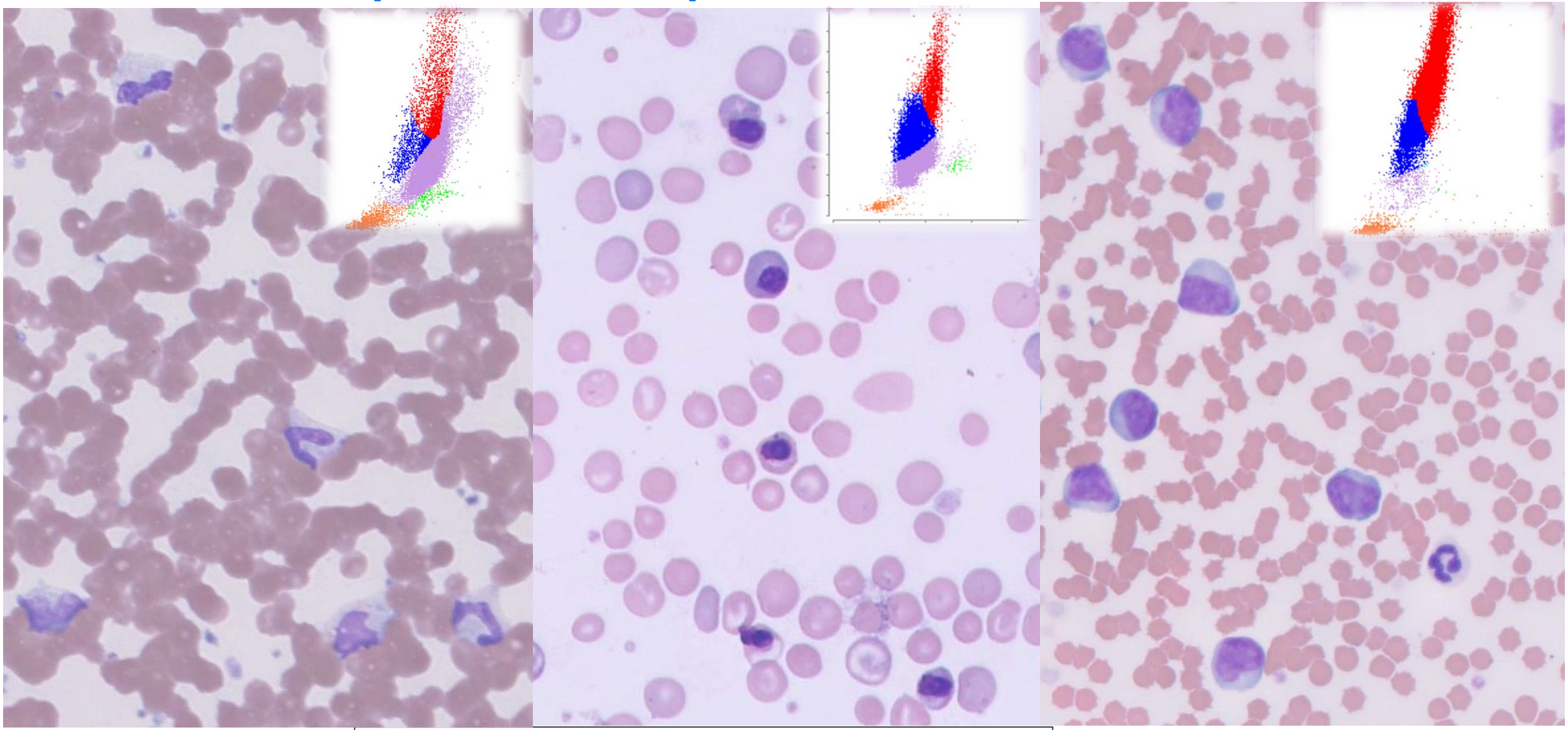








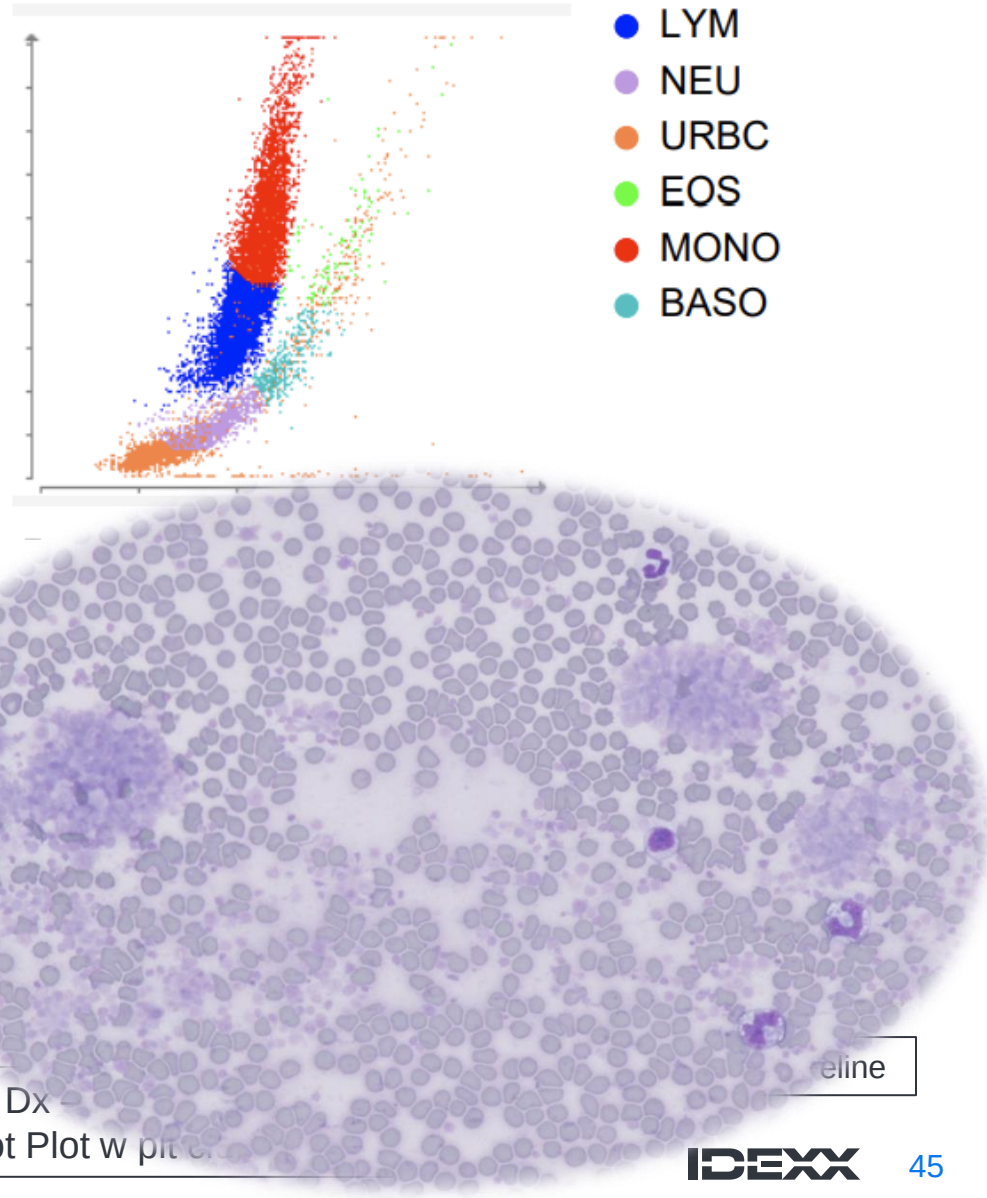
White blood cell abnormalities…
When the dot plots don't help



ProCyte Dx – Canine Patient WBC Dot Plots and respective blood smears

Case 5 – Nefeli – What else may be happening …

WBC	***17.78**	2.87 - 17.02 x10^9/L
% Neutrophils	*8.3	%
% Lymphocytes	*46.2	%
% Monocytes	*42.7	%
% Eosinophils	*0.7	%
% Basophils	- --.--	%
Neutrophils	***1.47**	2.30 - 10.29 x10^9/L
Bands	*Suspected	
Lymphocytes	***8.21**	0.92 - 6.88 x10^9/L
Monocytes	***7.60**	0.05 - 0.67 x10^9/L
Eosinophils	***0.12**	0.17 - 1.57 x10^9/L
Basophils	- --.--	0.01 - 0.26 x10^9/L
Platelets	***74**	151 - 600 x10^9/L
MPV	*14.5	11.4 - 21.6 fL
Plateletcrit	***0.11**	0.17 - 0.86 %

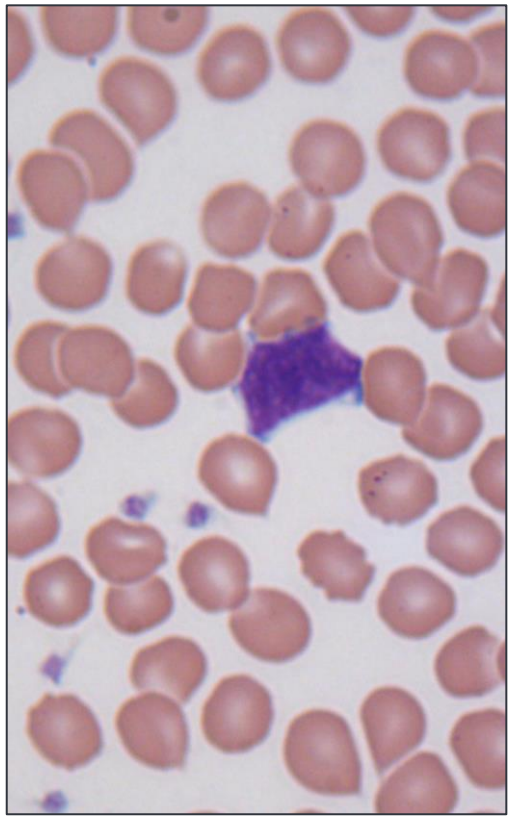


ProCyte Dx
WBC Dot Plot w pit c

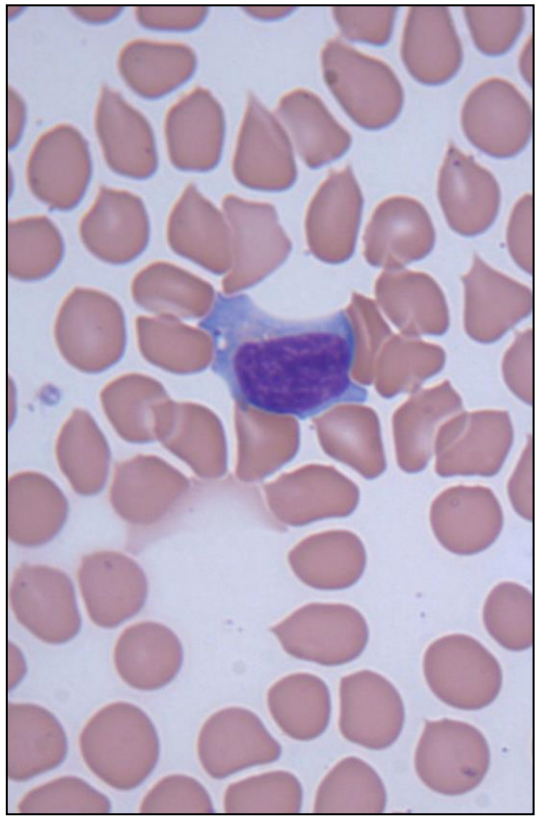
eline

Lymphocytes morphological assessment

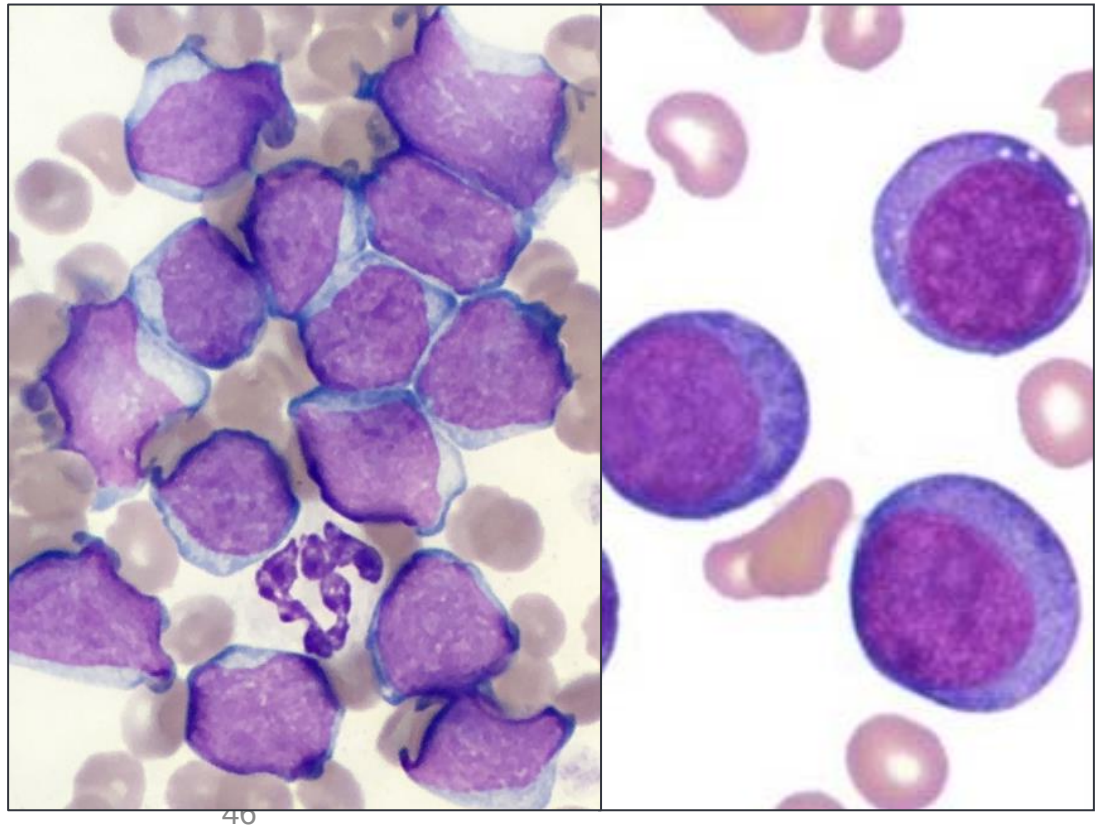
Normal Lymphocyte



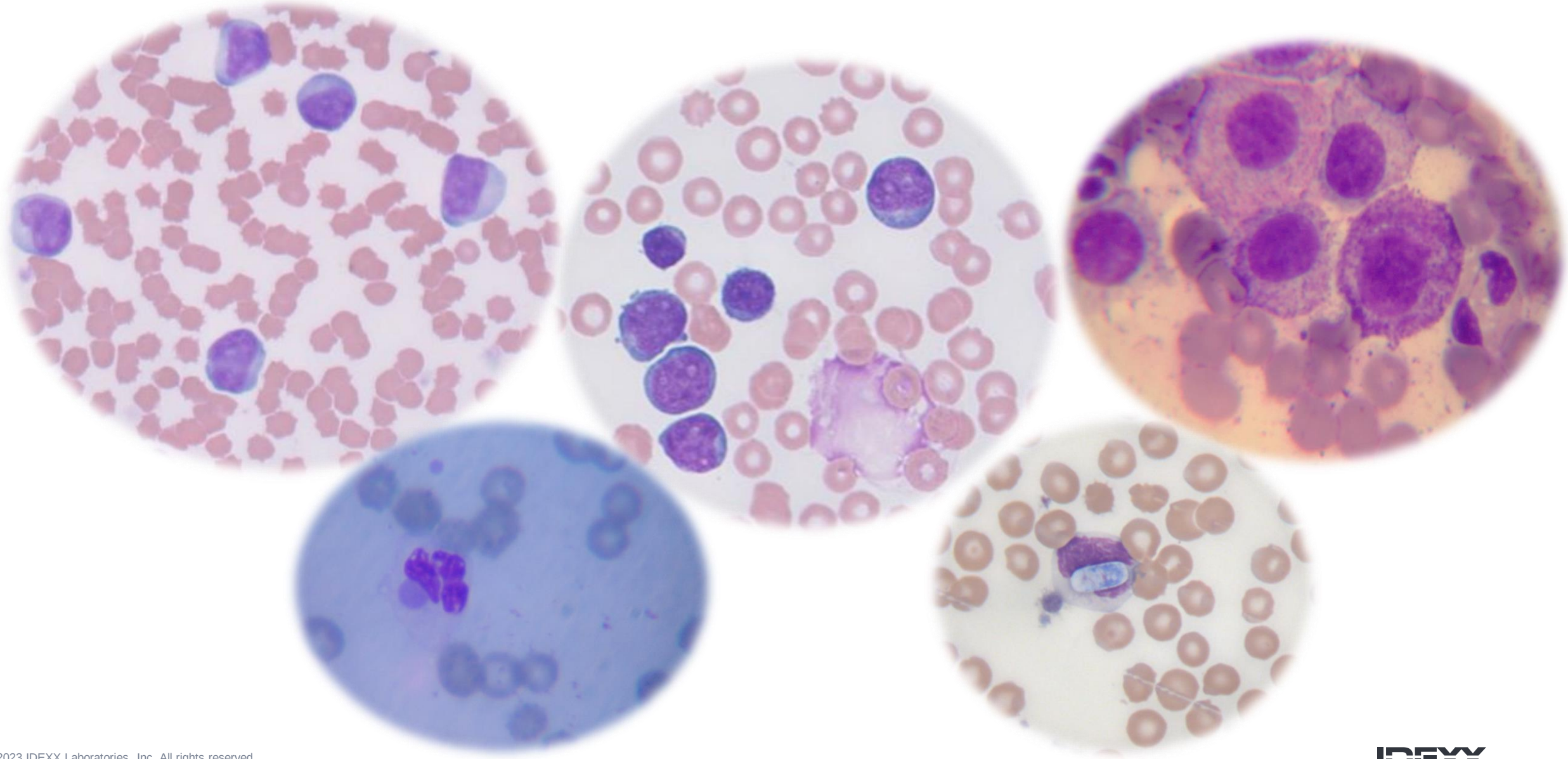
Reactive Lymphocyte



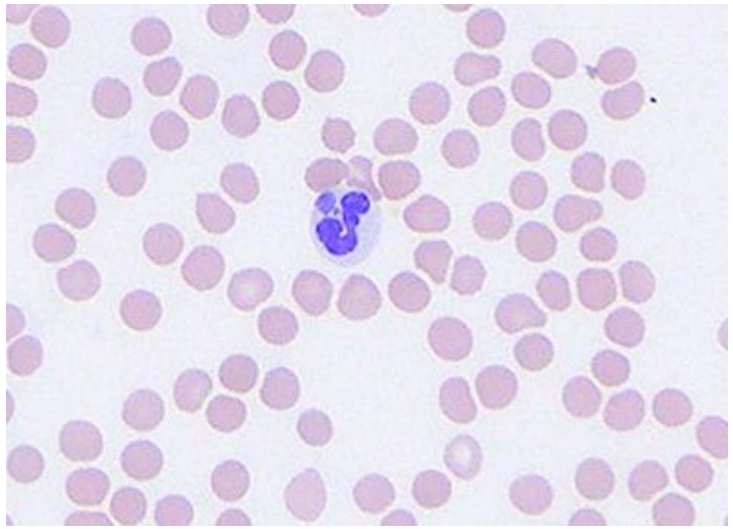
Atypical cells/blasts

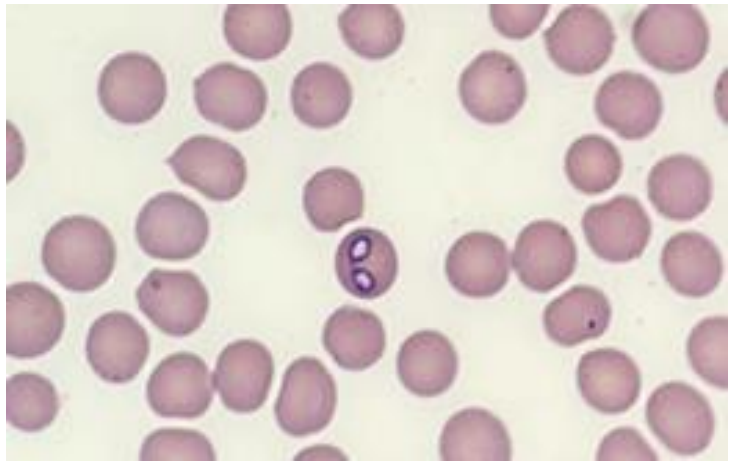


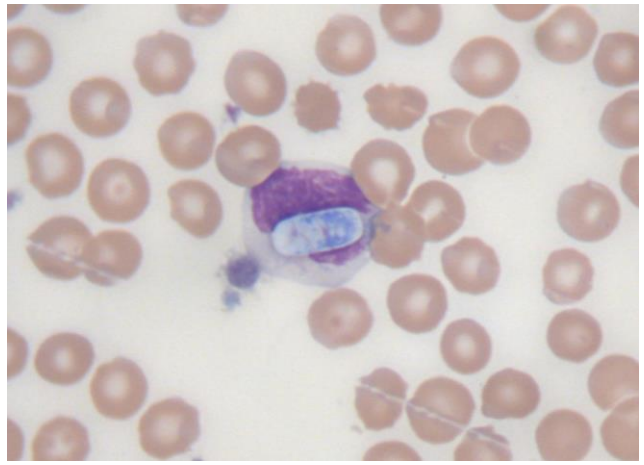
Inclusions, atypical cells and blasts



Infectious agents







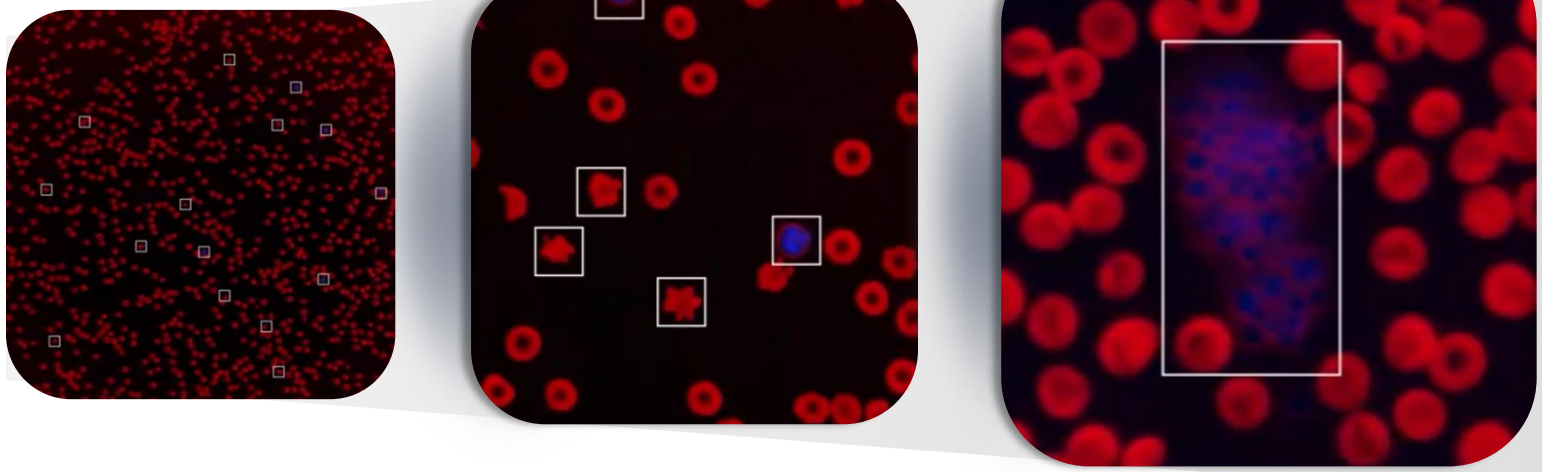
Take home MSG

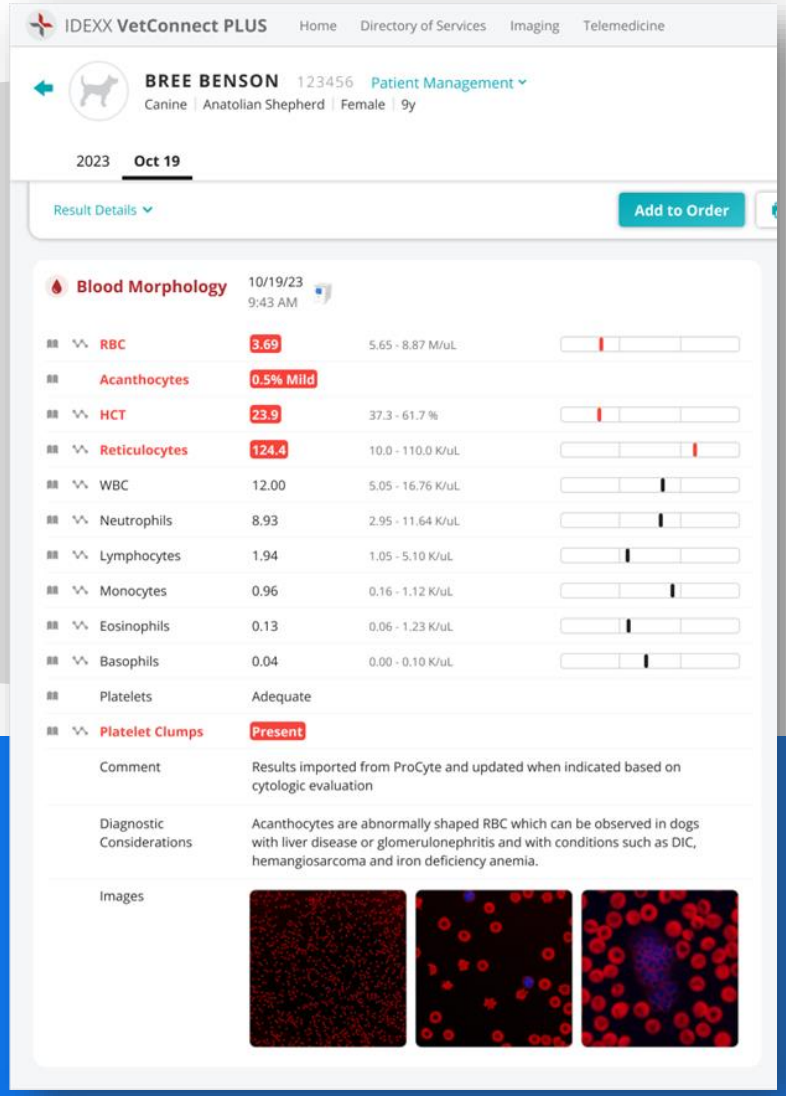
- Even with best analyzers

  Blood smear exam allows you to get more out of your CBC results

- Start with a good quality smear
- Have a systematic approach
- Cross check findings with analyzer and history
- If in doubt… refer…get us to help out!
- **A different workflow to blood morphology evaluation is in the cards**

IDEXX inVue Dx™ analyzer: automated quantification, classification, and interpretation of blood morphology

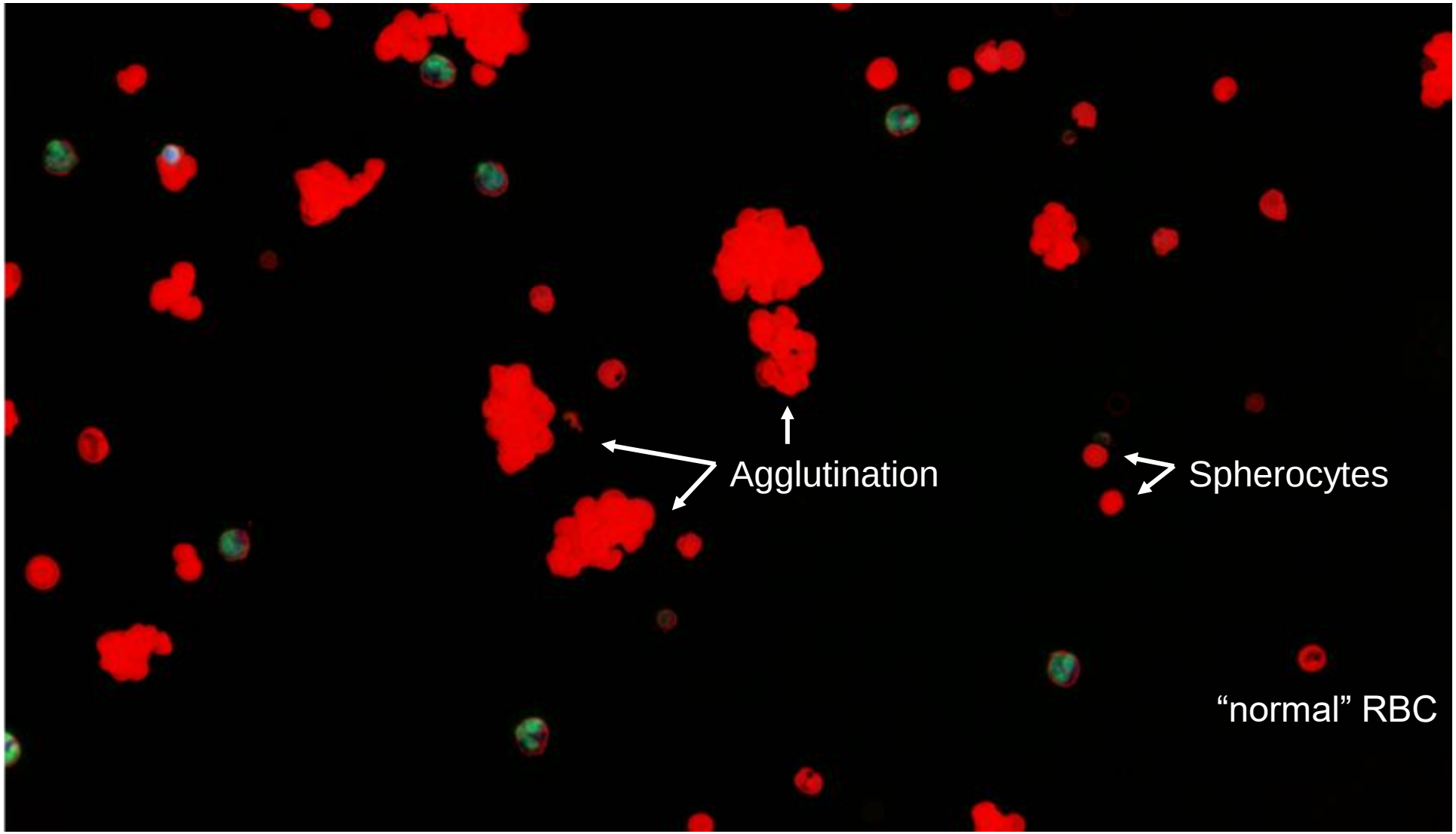


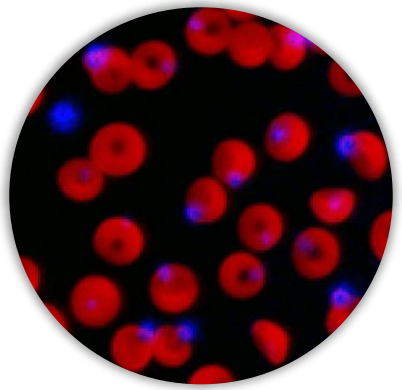


Results

in **10 minutes**

IDEXX inVue Dx™ analyzer: blood morphology image





IDEXX inVue Dx™ analyzer: Integrated report

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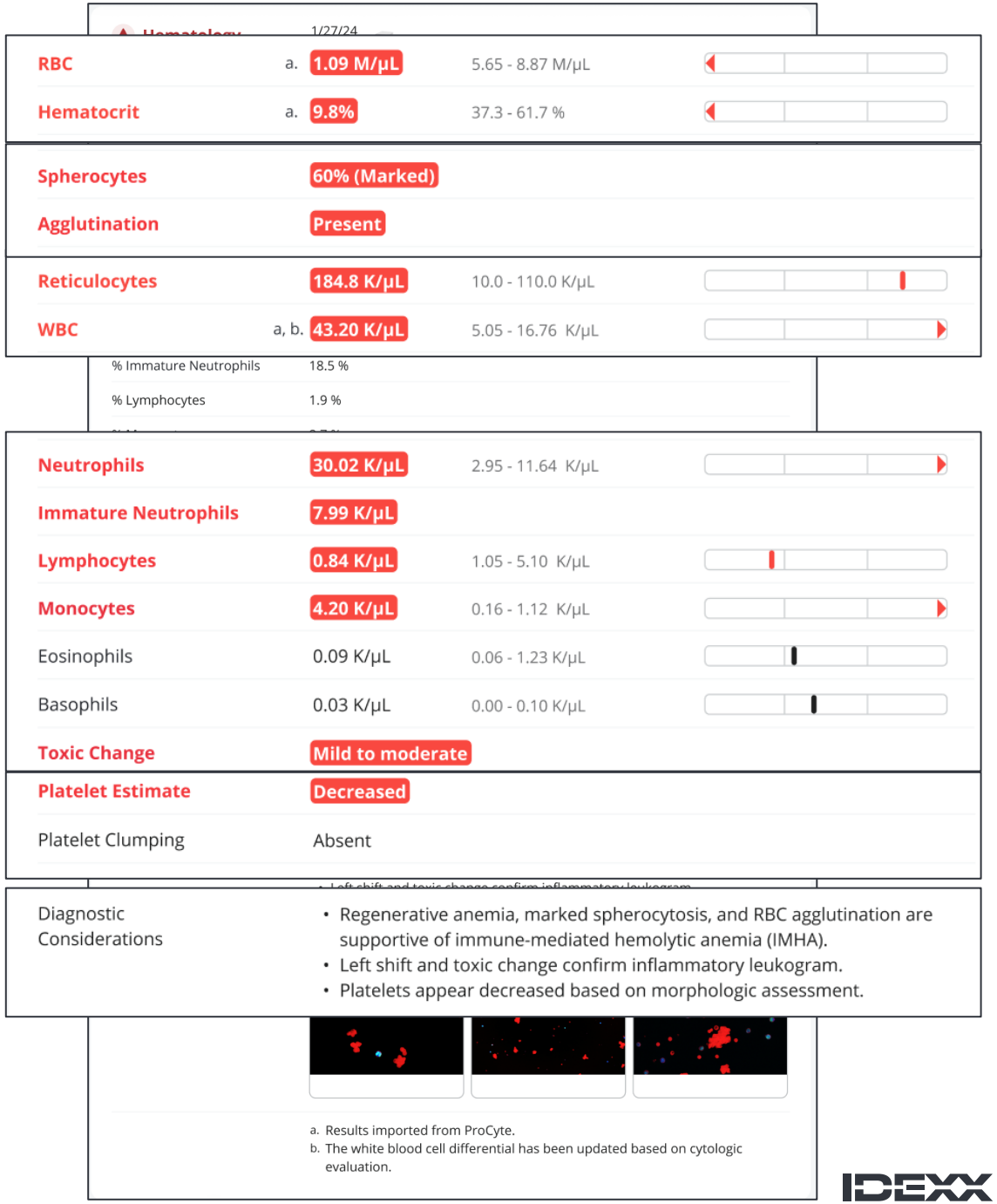

Thank you!
Any questions?

Questions?

References:

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